ETIOLOGY AND CONTROL OF BULB ROT DISEASE OF ONION (*Allium cepae* L.) IN THE EASTERN REGION OF GHANA

BY

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DECLARATION

I, Adam Iddrisu Alidu, author of this thesis do hereby declare that except for the references to other peoples' work which have been duly cited, this thesis is the result of my own findings under the supervision of Professor S. K. Offei and Dr. E.W. Conerlius. I also declare that the work has neither in whole or part been presented for another degree in the University of Ghana or elsewhere.

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(CO-SUPERVISOR) DATE…………………………..

DEDICATION

'THE KEY TO SUCCERS IS HARDWORK'

I therefore dedicate this work to my family and friends.

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LIST OF ABBREVIATIONS

AEAs	Agriculture Extension Agents
AFLP	Amplified Fragment Length Polymorphism
AUDPC	Area Under Disease Progress Curve
DADU	District Agricultural Development Unit
FBR	*Fusarium* basal rot
FOC	*Fusarium oxysporium* f. sp. *cepae*
f sp	Formae specialis
GSG	Garcia Science Center
ISTA	International Seed Testing Association
MoFA	Ministry of Food and Agriculture
NaOCl	Sodium Hypochlorite
NMU	New Mexico State University
PCR	Polymerase chain reaction
PDA	Potassium Dextrose Agar
RAPT	Random Amplified Polymorphic Technique
PPRSD	Plant Protection and Regulatory Services Directorate
SARI	Savanna Agricultural Research Institute
SINNA	Research garden of the Crop Science Dept of, UG
ULV	Ultra Low Volume
UG	University of Ghana
WP	Wettable powder

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ABSTRACT

A study was carried out to assess farmers' knowledge on prevalence, spread, control and economic importance of onion bulb rot disease in the Fanteakwa and Kwahu South Districts in the Eastern region of Ghana, determine the incidence and severity of the disease in the two districts and identify the causal organism of the disease and to develop a chemical control regime for management of the disease. Some local and exotic onion lines were screened for resistance to the disease. Eighty farmers were randomly selected from the experimental sites and interviewed using pretested semi-structured questionnaires. The questionnaires focused on the background of farmers, their knowledge and experiences on prevalence, spread, control and economic importance of the disease. Disease incidence was determined by counting the number of both healthy and diseased onion plants and disease severity was scored using a one to five disease assessment key on five onion farms in each operational area. The incidence and severity of the disease was higher in the Kwahu south district than the Fanteakwa district. *Fusarium* f. sp. *cepae* was consistently isolated from diseased samples brought from the field using morphological characteristics and PCR detection of the genomic DNA from the isolate. Four antagonistic agents comprising of two biological agents namely *Trichoderma asperellium* and *Bacillus subtilis* and two synthetic chemicals (Carbendazim and Mancozeb) were assayed on PDA against *Fusarium oxysporum* f. sp. *cepae.* The two synthetic fungicides resulted in 100% inhibition of mycelial growth of the fungus. *Trichodema asperelium* induced 76.5% inhibition while the *bacillus subtilis* resulted in 20% inhibition of mycelial growth after six days of incubation. Bawku Red produced the highest seedling emergence rate of 72.16% and the highest seedling survival rate of 65.70% following inoculation with the fungus in the screening obbays. Texas Grano had the lowest seedling emergence and survival rates. Mature plant screening was conducted on three cultivars (Bawku Red, Malavi and Red Creole). The least affected variety was Malavi with lower disease incidence (54.15%) severity (1.57) and also produced bigger bulbs compared to Bawku Red and Red Creole. The most affected was Bawku Red which also produced smaller bulbs as compared to the others. The controls were not affected with mean bulb weight of not less than 42 g for all the varieties. The efficacy of four antagonistic agents namely Carbendazim, Mancozeb, *Trichoderma asperellum* and *Bacillus subtilis* were evaluated against *Fusarium oxysporium* f. sp *cepae*. Malavi, a susceptible variety identified from the seedling screening was used as the experimental material in inoculated soil. Carbendazim was most effective fungicide. *Bacillus subtilis* was found to be least effective with disease incidence of 22.2% and severity rate of 1.2 recorded on onions treated with the organism. For management of the disease, Carbendazim or Mancozeb treatment at nursery, transplanting and spraying at four and eight weeks before harvest could be used alongside farm sanitation.

CHAPTER ONE

1.0 INTRODUCTION

Onion (*Allium cepae* L., Alliaceae) is a plant of economic importance and widely cultivated in the world (FAO 2005; Awuah *et al.*, 2009). It is a biennial vegetable grown as an annual worldwide. The cultivars that do well are annuals as they can produce seed within the first year of growing (Kyofa-Boamah *et al*., 2000).

The uses of onion date back to over 4,000 years beyond the beginnings of written history (Necola, 2007). Onions are used in salads, cooked, eaten raw and for soups. They are also used in traditional medicine for treatment of chicken pox, common cold, influenza, measles, and rheumatism (Schwartz and Mohan, 1995). Large sums of money are spent each year importing onions into Ghana from our Northern neighbors; about 120 tons/annum from Burkina Faso, (MoFA/PPRSD, 2012).

The crop is suited to smallholder farming in most countries in the tropics and can be grown all year round where irrigation is available (Necola, 2007). Commercial onion cultivation in Ghana is concentrated in the Northern and Upper Regions, Berekum in Brong Ahafo, Fanteakwa and Kwahu South in the Eastern region (Awuah *et al.*, 2009). However, onion-producing areas in the country have expanded to include Sogakope and Akatsi (Volta region), Ashaiman and Dawhenya (Greater Accra region), Nsawam (Eastern Region) and Prestea (Western Region), (Awuah *et al.*, 2009). Onion yields in the Northern/Upper and Southern regions of Ghana are 15-25 t/ha and 5-15 t/ha respectively (Norman, 1992). The most common cultivars of onion cultivated in Ghana are the Bawku Red, ‘Malavi’ and Red Creole but other exotic cultivars such as Texas early Grano, Crystal White Wax, Lisbon White and market Winner have also been reported to be suitable for cultivation (Awuah *et al.*, 2009).

Despite its importance, onion is susceptible to a number of diseases including Purple blotch, Botrytis leaf blight, Downy mildew, Soft rot, Pink rot, White rot and Onion twister (PANS, 1996; Oduro, 2000; Hicks, 2004; Koike *et al.*, 2007; APS, 2008; Offei *et al.*, 2008).

The current challenge of onion farmers in the Fanteakwa and the Kwahu south districts of the Eastern region of Ghana is the occurrence of a bulb rot disease, which threatens its production in the area. The disease is locally known as 'apropro' after its main symptom which is soft rot of the bulbs and also referred to as 'heart rot' by the Agricultural Extension Agents (AEAs) and is perceived to be the most important production constraint in the area. Some farmers in the area have abandoned onion cultivation because of the disease and gone into other commercial ventures such as fishing and petty-trading (Azidoke and Gyemfi, Personal Communication, 2010), which has resulted in reduced income and increased levels of poverty, school dropout and migration of the youth to urban centers. A similar disease was reported in the New Oworobong area in the Kwahu south district in the 1980s and in 2006 (Awuah *et al.*, 2009).

AEAs and farmers in the area have little knowledge about the causes of the disease and its control. In an attempt to control the disease, farmers misapply synthetic pesticides such as champion (Kocide/Cupric hydroxide), Nodox (Cuprous oxide), Funguran (Copper hydroxide) and Fungukill (Copper + Metalaxyl) which have not been effective in controlling the disease and have the potential of contaminating farm produce and the environment (Awuah *et al.*, 2009).

The objectives of this study were therefore to:

i. Conduct a field survey to assess farmers' knowledge on prevalence, spread, control and economic importance of the onion bulb rot disease in the Fanteakwa and Kwahu South Districts.

ii. Determine the incidence and severity of the bulb rot disease in the two districts.

iii. Identify the causal organism of the disease.

iv. Screen some local and exotic onion lines for host plant resistance to onion bulb rot disease.

v. Develop a chemical control regime for management of the disease.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Botany of onion

Onion (*Allium cepa* L.) is a member of the lily family (Alliaceae) (Necola, 2007). They have a diploid genome and are cool-season biennials, undertaking three development phases (leave formation, bulb formation and maturity) throughout the growing season (Kyofa-Boamah *et al*., 2000; FAO, 2005). The crop is tolerant to frost and produce bulbs with growing day lengths (short, intermediate and long day), (Necola, 2007). Onion cultivars are classified according to day length required for bulb formation, market use (green, fresh bulbs and dehydrated bulbs) bulb colour (such as Red, white, yellow and purple skin) and elongated or globe shaped (Kyofa-Boamah *et al*., 2000; Necola, 2007).

2.2. Importance of onion

Onion is a plant of economic importance and widely cultivated in the world (FAO, 2005; Awuah *et al*., 2009). It is an important vegetable crop (Sinnadurai, 1970; Kyofa-Boamah *et al*., 2000) whose distinctive flavor is appreciated by people throughout the world and therefore ranks fourth in world production of vegetable with a volume of 64.101 metric tons annually (FAO, 2005).

2.2.1 Food value of onion

Onions are chief food plants in which the food is stored in a bulb (Craig, 2010). The bulbs can be harvested and sold either 'green' in salads, while the mature bulbs are cooked or eaten raw as a vegetable (Craig, 2010). In general, onions are used for salads (bunching onion or sliced full-grown bulbs), pickling (for example, silver skin onions), cooking (such as in soups) and frying (for example, with meat). The per capital consumption of onion is approximately 4 kg per annum (Anonymous, 2005). Nutrient-wise 100 g of onion provides about 30 g of calcium, 0.5 mg of iron, vitamin B, 0.2 mg of riboflavin, 0.3 mg of nicotinamide, and 10 mg of ascorbic acid (Anonymous, 2005; Craig, 2010).

2.2.2 Medicinal Importance

Onion plays an important role in traditional medicine as a diuretic for treatment of chicken pox, common cold, influenza, measles, and rheumatism (Schwartz and Mohan, 1995). The antimicrobial characters of the alliums are likely the result of the effect of sulphur compounds (Schwartz and Mohan, 1995). Research has demonstrated that extracts of onion decrease sugars, lipids, and platelet aggregation and enhances fibrinolysis in the blood, indicating that the alliums may help to prevent arteriosclerosis and other cardiovascular diseases (Schwartz and Mohan, 1995).

2.3 Onion Cultivation in Ghana

Onion producing areas in southern Ghana include Sogakokpe and Akatsi in the Volta region, Ashaiman and Dawhenya in the Greater Accra, Nsawam in the Eastern region and Prestea in the Western Region (Awuah *et al.*, 2009). In the Upper East region of Ghana, approximately 15,000 farming families were active in onion production in year 2010 at Bawku West and Garu Tempane districts (Trias, 2010). Onion yields in the Northern/Upper and Southern regions of Ghana are 15-25t/ha and 5-15t/ha respectively (Norman, 1992).

The most common onion cultivars cultivated in Ghana are the local red, short day cultivars, Bawku Red, 'Malavi' and Red Creole. Other exotic cultivars such as Texas early Grano, Crystal White Wax, Lisbon White, Yellow Flat, Suttons, Australian Brown, Early Cape and Market Winner were in the past introduced into the country (Kyofa-Boamah *et al*., 2000; Awuah *et al*., 2009). Cultivars that performed best (Texas Early Grano, Red Creole, Market Winner and Australian Brown) have been maintained to date (Awuah *et al.*, 2009).

2.4 Pest and diseases of onion

Several pests and diseases affect the onion plant worldwide (Kyofa-Boama *et al*. 2000; Corgan, 2002). The diseases include Pink root (*Pyrenochaeta terrestris*), *Fusarium* basal rot (*Fusarium oxysporium*), Onion smut (*Urocystis sepulae*), Mould (*Aspergillus* spp.), Botrytis bulb rot (*Botrytis* spp.), Iris yellow spot virus, and bacterial soft rots (*Pectobacterium* spp. *Dickeya*, and *Burkholderia* spp.). Some of the onion pests are Blister beetle (*Epicautu blineata*), Leaf worm (*Spodoptera litoralis*), Onion fly (*Delia antique*), Leaf miners (*Phytobia cepae*), Cut worm (*Agroitis* spp.), Root knot nematodes (*Meloidogyne* spp.) and thrips (*Thrips tabaci* L.). (Walker *et al.*, 2009; Corgan, 2002; Khatun and Ashrafuzzaman, 2008). Some important onion diseases in Ghana are shown in Table 1.

Table 1: Common diseases of onion reported in Ghana

Disease	Type of Infection	Causal Agent	Symptom
Basal rot	Fungus	*Fusarium* spp.	Bulb rot, die-back, wilt and pink roots.
Bulb rot	Fungus	*Sclerotium rolfsii*	Leaf bases of bulbs attacked causing rot.
Downy mildew	Fungus	*Peronospora destructor*	Pale-green or brownish elongated lesions on leaves and bulb, leave die back.
Onion twister	Fungus	*Colletotrichum gloeosporiodes*	Twisting, curling and yellowing of leaves, lesions on leaves.
Purple blotch	Fungus	*Alternaria porri*	Tip of leaves dry out, and covered with black spores and purplish centre produced lower down the leaf.
Root knot	Nematode	*Meloidogyne* spp.	Widening of the roots at irregular intervals, but in all of them the Plant eventually dies.

Modified after Offei *et al* (2008)

Fusarium basal rot (FBR) is the second most important soil borne disease affecting onion crop worldwide (Cramer, 2002). It causes disease and losses throughout the life cycle of the crop from seed germination to seed production (Awuah *et al.*, 2009; Saxena and Cramer, 2009) with disease incidence of 5.0 – 63.6% in New Mexico (Cramer *et al.*, 2003). Oduro (2000) reported onion bulb rot disease in Ghana to be caused by *Fusarium oxysporium* f. sp. *cepae*. All onion production areas in Ghana encounter the onion bulb rot disease caused by *Fusariun oxysporium* f. sp. *cepae* indicating its importance (Awuah *et al.*, 2009). The disease causes remarkable economic losses to onion farmers globally (60%) (Cramer, 2002). Once infested with *Fusariun* basal rot disease, the bulbs become unmarketable (Corgan, 2002; Cramer, 2002). Awuah *et al.* (2009) reported total lost in the new Oworobong area of the Kwahu South district in the Eastern Region of Ghana where onion cultivation is most intensive.

2.5 Causal Agents of Fungal diseases in onion

Four different species of *Fusarium* have been observed to cause disease in onions (Sara, 2010) (Table 2). However, it is not fully understood to what extent *F. avenaceum* causes disease in the crop, or if the onion merely serves as an alternative host (Sara, 2010).

Table 2: Species of *Fusarium* infecting onions

***Fusarium* spp.**	**Disease**	**Reference**
Fusarium oxysporium	Fusarium basal plate rot	Schwartz and Mohan (1995), Awuah *et al.* (2009)
Fusarium redolens	*Fusarium* basal plate rot	Booth (1971), Shinmura (2002)
Fusarium culmorum	*Fusarium* basal plate rot	Galván *et al.* (2008)
Fusarium avenaceum	Unknown	Galván *et al.* (2008)

Source: Sara (2010).

Fusarium oxysporium f.sp. *cepae* occurs worldwide (Abawi and Lorbeer, 1972). Causing basal rot disease in a number of *Allium* species such as onion, chive, garlic and shallot (Dugan *et al.*, 2007). The pathogen is a Deuteromycete and has no known teleomorphic stage (Brayford, 1996; Agrios, 2005; Galván, 2009). The fungus produces mycelium as well as three types of asexual spores namely microconidia, macroconidia and chlamydospores (Cramer, 2000; Howard *et al.*, 2007).

Microconidia are the most commonly produced spores and are 5-12 µm in length. They are typically without septa and their shape varies from oval to kidney shaped. Macroconidia have a characteristic falcate shape making them easily identifiable. In addition, they typically have three or four septa (Howard *et al*, 2007; Galván, 2009).

Chlamydospores are produced in or on older mycelium, have one or two round cells and have thick cell walls, which defend the cells against degradation and antagonists. It helps the fungus to survive in the soil in the absence of its host for a very long time, usually indefinitely. *Fusarium* species can survive either as mycelium or as spores on plant debris in the soil (Howard *et al*, 2007; Galván, 2009). The fungus can disperse with soil particles and plant debris, which can be transported by both water and farm equipment (Cramer, 2000), and can survive without any sexual stage during its entire life cycle (Swift *et al*., 2002; Agrios, 2005; Howard *et al*, 2007).

2.6 Infection of onion by *Fusarium oxysporium* f.sp. *cepae*

The infection of onion by *Fusarium oxysporium* f.sp. *cepae* commences when the crop comes into contact with the fungus, usually via the soil (Abawi and Lorbeer, 1971; Howard *et al.*, 2007).

Chlorosis of all the leaves is the first above ground symptom observed on diseased plants (Schwartz and Mohan, 1995; Cramer, 2002). Tip necrosis closely follows chlorosis, which gradually spreads to entire leaves (Brayford, 1996, Lopez and Cramer 2004). Other preliminary symptoms consist of curling, wilting and yellowing of onion leaves (Sumner, 1995). Within the basal plate, the fungus causes a brown discolouration of the basal plate tissue resulting in the rotting of roots and death of the plant (Offei *et al*., 2008).

Fusarium basal rot of onion can also delay seedling emergence (Jessica *et al.*, 2006), and cause seedling damping off (Srivastava and Qadri, 1984; Saxena and Cramer, 2009), and stunted growth of seedlings (Jessica *et al.*, 2006). Infection within the basal plate also causes root death and root abscission (Cramer, 2000). A diagnostic symptom of the disease is the separation of roots from the bulb at the stem during uprooting (Tahvonen, 1981; Cramer, 2000).

The fungus develops when the soil temperature is between 15 to 32 °C (Jessica *et al.*, 2006; Schwartz, 2010). Studies here show that there is almost no disease when the soil temperature drops below 12 °C (Jessica *et al.*, 2006). The optimum temperature for its development is 25-32 °C (Schwartz and Mohan, 1995; Howard *et al.*, 2007). *Fusarium* basal rot disease severity and incidence vary from one year to another (Gutierrez and Cramer, 2005); hence it is difficult to predict losses on a regular basis. Disease severity is influenced by climate, crop rotations, drying, storage conditions, and disease control measures (Howard *et al.*, 2007), however most onion diseases begin on plants growing in the field and continue to develop on the bulbs during storage and transit (Walker *et al.*, 2009; Joon *et al.*, 2000; Khatun and

Ashrafuzzaman, 2008). Mechanical damage and wounds caused by other organisms assist the process of infection especially if the soil is infected (Khatun and Ashrafuzzaman, 2008).

Somkuwar *et al.* (1996), Stadnik and Dhingra (1996), Cramer (2000), Awuah *et al.* (2009) and Sara (2010) consistently isolated *Fusarium* from rotten onion bulbs in India, Brazil, New Mexico, Ghana and Sweden respectively. Colonies were identified as *F. oxysporium* f. sp. *cepae* based on morphological characteristics.

Some investigators have reported that *Fusarium oxysporium* f. sp. *cepae* attacks onion only as wound parasites (Jessica *et al.*, 2006). Rajapakse and Edirimanna (2002) infected onion bulbs by making needle-prick inoculations with *Fusarium* sp. isolated from diseased onion plants, and in studies in Iowa it was found that *F. oxysporium* f. sp. *cepae* attacks onions only after artificial wounding. When combined with *Phoma terrestris*, *Fusarium* became a virulent secondary invader and caused a semi-dry rot (Schwartz and Mohan, 1995; Jessica *et al.*, 2006).

Direct penetration of onion by *Fusarium* has been reported (Jessica *et al.*, 2006). Differences in pathogenicity and virulence of different isolates of *Fusarium* have been observed using a set of cultivars of onion, Garlic Seed and Cloves (Cramer, 2002; Jessica *et al.*, 2006; Galván, 2009) and the differences were attributed to vertical resistances of individual cultivar lines. The disease can be very damaging to susceptible cultivars in fields with a history of *Fusarium basal rot* (Howard *et al.*, 2007).

2.7 Management of *Fusarium* basal rot disease

Several crop management procedures have been undertaken to prevent the spread and infection of onion by *Fusarium* basal rot fungus, including the use of resistant onion cultivars, biological control agents, crop rotation, solarization, regular cleaning of equipment, dipping seedlings in fungicide, fumigation of the soil and Integrated Pest Management (IPM) practices (Schwartz and Mohan, 1995; Cramer, 2000; Offei *et al.*, 2008; Walker *et al.,* 2009).

2.7.1 Biological control agents

Biological control using fungal and bacterial antagonists has been suggested as a possible control of *Fusarium* basal rot disease of onion (FBR) (Cosskuntuna and Ozer, 2008). In an *in-vitro* test, Zlata *et al.* (2008) observed that, a strain of *Trichoderma asperellum* was able to overgrow on isolates of *F. solani* mycelia and produce conidia on its surface to indicate mycoparasitic action.

Trichoderma harzianum inhibited mycelial growth of *F. oxysporium* f. sp. *cepae* in an *in-vitro* test and reduced disease incidence comparable to imidazole fungicide in a pot culture experiment (Cosskuntuna and Ozer, 2008). A combination of *Trichodema* sp. and *Pseudomonas fluorescents* were most effective for reducing incidence of FBR under pot and field conditions (Rajendran and Ranganathan, 1996).

Among several biological control agents tested against onion bulb rot on field and in storage *Trichoderma harzianum* and *Bacillus amyloliquefaciens* were found to be most effective both on field and in storage, suggesting that biological control of postharvest decay of onion using these microorganisms either at the time of transplanting or at harvesting may be promising (Joon *et al*., 2000). When the antagonists were applied to the topping areas of onion bulbs at harvest *Trichoderma harzianum* was the most effective biological control agent against basal rot, with the number of rotten bulbs recorded at 4%, while that of the control was 16%.(Joon *et al*., 2000).

2.7.2 Fungicides

Thiophanate–methyl 50% WP, Thiram 30% WP, Chlorothalonil 70% WP and Carbendazim 50% WP were tested against FBR in-vitro and Carbendazim was found to be most effective (Rajapakse and Edimanna, 2002). Carbendazim 50% WP applied at the rate of 1.4g/L, helped reduced field rotting losses as well as storage losses of onion bulbs by 35 and 40 percent respectively (Dugan *et al.* 2007). Carbendazim at 0.2 per cent was the best treatment amongst the fungicides tested against *Fusarium oxysporium* sp when combined with *T. harzianum* (Rajendran and Ranganathan, 1996).

Sunita and Katoch (2001) studied the comparative efficacy of systemic, protectants and miscellaneous fungicides *in-vitro* against *Fusarium oxysporium* f. s*p*. *Dianthi* causing wilt of carnation. Carbendazim and Benomyl completely inhibited the growth of test fungus *in-vitro* (Sunita and Katoch, 2001). Even under field conditions Carbendazim and Benomyl reduced the wilt incidence up to 80% (Sunita and Katoch, 2001). Among the systemic fungicides, Carbendazim was highly effective in inhibiting the growth of *Fusarium oxysporium* f. sp. *cepae* in all the three concentrations (0.025%, 0.05% and 0.1%) tested (Dugan *et al.* 2007). Among the protectant fungicides, Mancozeb at 0.3 per cent performed better than other fungicides in inhibiting *Fusarium oxysporium* f. sp. *gladioli* (Kulkarni, 2006).

2.7.3 Crop rotation

Four years crop rotation with maize or wheat reduced soil inoculum levels and subsequently onion bulb loss to FBR by over 90% (Higashida *et al*., 1982). A crop rotation of four years with a nonsusceptible host is recommended before planting another onion crop in that field (Higashida *et al*., 1982; Entwistle, 1990; Havey, 1995).

2.7.4 Solarisation

Field solarisation has been reported to decrease the incidence of *Fusarium* basal rot diseases (Katan *et al*., 1980). Awuah *et al.* (2009) suggested that burning of thresh on seedbeds to obtain a sterilizing effect on soil may help in production of disease free onion seedlings, free from *Fusarium* basal rot disease caused by *Fusarium oxysporium* f sp *cepae* a soil borne fungi.

2.8 Screening of onion cultivars for resistance to *Fusarium* basal rot disease

In order to develop or select resistant cultivars to *Fusarium* bulb rot disease, numerous screening methods have been developed that include field or greenhouse seedling screening, mature plants screening and dormant bulb screening (Cramer, 2000; Lopez, 2003; Jessica *et al*., 2006). Lopez and Cramer (2004), screened forty-two short-day onion germplasm inoculated with 4.0 x 10^4 spores per gram of sand for FBR resistance using inoculated seedling alongside the mature bulb screening methods. In the seedling study 'NuMex Luna' and NMSU 00-32 varieties had the highest seedling survival rate (23.6% and 20.4% respectively), and in the field study, 'Cardinal' was found to have the highest FBR incidence (90.8%) and severity (4.9).

'NMSU 00-13-1' was found to have the lowest FBR incidence (10%) and severity (1.11). A strong, positive correlation existed between FBR incidence and severity, but were not correlated with seedling survival. The ideal inoculum concentration for seedling screening procedure was found to be 1.0 X 10^4 spores per gram of sand (Lopez, 2003), while ideal concentration of spores in conidial solution is 10^5 conidia per ml of water (Apaza and Mattos, 2000). In a related work, Jessica *et al.* (2006) screened 22 winter-sown onion germplasm for FBR resistance using a matured bulb field screening at harvest and four in storage for 2 years. NMSU 99-32, NuMex Aurthur and NuMex Jose Fernandez were found to have the lowest disease severity and incidence in both years (Jessica *et al.*, 2006).

Onion has a longer duration for screening of resistant cultivars against diseases under field conditions as compared to other annual crops (Cramer, 2000). It is therefore necessary to develop other rapid technique for screening (Lopez, 2003). Seedling screening procedure is a perfect tool that could be reliably exploited for breeding against *Fusarium* bulb rot (Retig *et al.*, 1970). Sokhi *et al*. (1974) suggested that testing at bulbing stage gives more accurate response to selection. Moreover Stadnik and Dhingra, (1996) suggested that determination of true tolerance will be possible only at harvest through yield loss. A total of 136 combinations of FOC isolates and onion cultivars were evaluated at 25°C for 20 days for two years,'NuMex Chaco' and 'NuMex Crispy' were found to have the highest FBR incidence whiles FOC isolate 'GSCG 515' were the best isolates recommended for future screenings (Saxena and Cramer, 2009). It has been suggested that, seedling and mature plant screening will provide more valid and reliable results (Lopez and Cramer, 2004). Stadnik and Dhingra (1995) however, found field screening procedures not to be feasible due to longer time duration requirements and inconsistent results, and that the variation in results of field screening was due to seasonal variations (Apaza *et al.*, 2000). More than one type of resistance mechanism has been found to be present in onions, which become active at different crop developmental phases (young seedlings, older plant and mature bulb) (Galván, 2009).

2.8.1 Host plant specificity in Fusarium oxysporium formae specialis

Differentiation of species into *formae specialis* (f. sp.) has been useful to Plant Breeders and Plant Pathologists because it comprises a subset of isolates which attacks only specific host plant (Gordon and Martyn, 1997; Cramer, 2000). Within *Fusarium formae specialis,* pathogenic variation exist and isolates attack only few cultivars of the host species (Cramer, 2000, Galván *et al.*, 2008; Feng *et al.*, 2010). *Fusarium oxysporium* is one of the most genetically diverse species of the genus *Fusarium* with over 120 different *formae specialis* (Bayraktar and Dolar, 2011). Table 3 shows some formae specialis of *Fusarium oxysporium*.

2.8.2 Onion cultivar resistant to Fusarium basal rot disease

Resistant cultivars of onions have sometimes shown to be susceptible and vice versa, due to variability in isolates of *Fusarium oxysporium* f. sp. *Cepae* used in trials (Saxena and Cramer 2009; Somkuwar *et al*. 1996). The age of seedling also has an effect on the resistance levels of genotypes (Badino *et al.*, 1980; Galván, 2009). In spite of these, onion cultivars found to be consistently resistant to FBR include NMSU 99-32, Nome Arthur, Utopia and Nome Jose Fernandez (Cramer, 2000; Lopez and Cramer, 2004; Jessica *et al.*, 2006).

Table 3: Some formae specialis of *Fusarium oxysporium* and the diseases they cause

Formae specialis of F. *oxysporium*	Disease/Host plant	Reference
F. oxysporium f. sp. *apii*	*Fusarium* yellows of celery	Lori *et al.* (2008)
F. oxysporium f.sp. *cepae*	*Fusarium* basal plate rot of onion	Schwartz and Mohan (1995)
F.oxysporium f. sp. *cubense*	*Fusarium* wilt of banana	Schwartz and Mohan (1995)
F. oxysporium f. sp. *cucumerinum*	*Fusarium* wilt of cucumber	Jenkins and Werner (1983)
F. oxysporium f. sp. *lycopersici*	*Fusarium* wilt of tomato	Agrios (2005)
F. oxysporium f. sp. *niveum*	*Fusarium* wilt of watermelon	Gordon *et al.* (1997)

Source: (Sara, 2010)

2.9 Socioeconomic background of the study area

The study was conducted in the Fanteakwa and Kwahu South districts in the Eastern region of Ghana where onion is commercially cultivated. Plate 1 is a map showing the location of the study site in the Eastern Region.

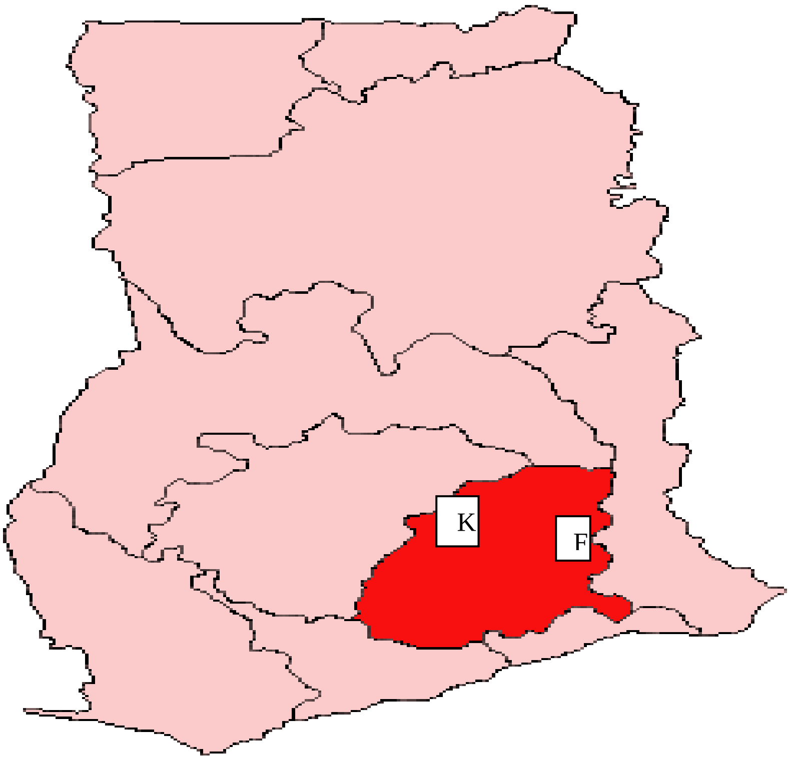

Plate 1: Map of Ghana showing Eastern region (Red), Fanteakwa (F) and Kwahu south districts (K)

2.9.1 The Fanteakwa District

Fanteakwa lies within longitudes 0°32.5' West and 0°10' East and latitudes 6° 15' North and 6° 40' North. Begoro is the District capital with an estimated population of 23,070 (18,200 in 1984) while the total District population stands at 103,711 (1997). It is bounded to the North by the Volta Lake, to the North-West by Kwahu-South District, South-West by the East Akim Municipal, Lower Manya Krobo District to the East and to the South East by the Yilo Krobo District. With a total land area of 1150 km^2, Fanteakwa District occupies 7.68% of the total land area within the Region (18310 km^2) and constitutes 0.48% of the total land area in Ghana. Total land area under cultivation is 362 hectares. Agricultural production is mainly at subsistence level with an average farm size of 2.5 acres owned by a family. Less than 30% of the farmers have access to extension services. The district does well in food and cash crop production as compared to the national output (tons per hectare) with exception of yam which lags behind. Yield for some selected crops include maize (69.40 t/ha), cassava (70.83 t/ha), vegetables (including onions) (16.87 t/ha) (Anonymous, 2005).

2.9.2 The Kwahu South District

The District is located in the northwestern part of the Eastern Region. It shares common boundaries with Sekyere East District to the north, Asante-Akim North Municipal and Asante Akim South District to the West, Kwahu West District to the East and Birim North, East Akim Municipal and Fanteakwa District to the South. Specifically, it lies between latitude 6^{o} 30’ N and 7^{o} N and longitude 0^{o} 30’ W and 1^{o} W. It covers a total land area of 1,462 square kilometers. Agriculture which is the predominant subsistence occupation in the district employs about 50.4% of the total labour force. Major crops cultivated by farmers include maize, cassava, groundnuts, vegetables (including onions), oil palm, plantain, cocoyam, citrus, cola and coffee. Major livestock and poultry produced are cattle, sheep, goats, pigs, grass cutters, fowls, ducks, turkeys and guinea fowls. Some local farmers are also engaged in inland fishing along the Afram River. A few farmers are engage in irrigated farming. The service sector is the next biggest employer in the district, engaging about 42.2% while industry, which is still in its teething stage, occupies only 7.4% of the labour force (Anonymous, 2005).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1.1 Selection of experimental sites within the two districts in the Eastern region for survey on bulb rot disease of onion

Two districts (Fanteakwa and Kwahu South) where onion bulb rot disease is prevalent were purposively selected. Three operational areas (communities) were selected at random for the survey. District Agricultural Extension Agents (AEAs) assisted in the identification of a number of villages with high prevalence of the disease which was listed as clusters or primary units within the districts. Random sampling selection was then employed for selecting three communities in each district for the survey. Fifteen (15) onion farmers in each community were randomly selected from a list of onion farmers provided by the AEA's. Table 4 shows the district, communities and number of farmers selected. The methods used for gathering information were administration of structured and semi-structured questionnaires, interviews, collection of diseased plant materials, counting of diseased plants and photographing.

3.1.2 Assessment. of farmers' knowledge on prevalence, spread, control and economic importance of onion bulb rot disease in the Fanteakwa and Kwahu South Districts

The questionnaire administration, surveys and interviews were conducted in August 2011. The operational areas visited at Fanteakwa were Mpeamu, Ganyankope and Abuakwa. At Kwahu south the communities visited were Amartey, Manguasi and Mpeamu Quarters (Table 4)

Table 4: District, communities visited and number of farmers interviewed in each community

District	Communities/villages	No. of Farmers
Fanteakwa	Mpreamu	14
	Ganyankope	9
	Abukwa	17
Kwahu South	Amartey	15
	Manguasi	15
	Mpreamu Quarters	10

A total number of 80 farmers were randomly selected from the experimental sites and interviewed using pretested semi-structured questionnaires. The questionnaire focused on farmer's background and their knowledge, perception, and experiences concerning prevalence, spread, control and economic importance of onion bulb rot disease. The items in the questionnaires (Appendix 1) were read to the farmers and their responses recorded.

All data collected from the questionnaire were subjected to descriptive statistics for analysis. The Statistical Package for Social Sciences (SPSS) version 16.0 software was used in carrying out the analysis.

3.1.3 Symptoms of onion bulb rot diseases in the Eastern Region of Ghana

Farm visits were undertaken in the two districts and the various stages of development of symptoms on diseased onion plants were observed, recorded and photographed. Diseased plants were uprooted to observe symptoms and compared to the descriptions and pictures in the compendium of onion diseases (Schwartz and Mohan, 1995). Diseased samples were then taken to the plant pathology laboratory at the University of Ghana, Legon and stored in a refrigerator at 5 ^{o}C and used for the isolation and identification of the causal agent.

3.2 Determination of incidence and severity of basal rot disease of onion in the Fanteakwa and Kwahu south districts

The objective of this study was to determine the incidence and severity of the disease in the two districts to enable the identification of disease hot spots for further studies. On each farmer's field, pegs were used to demarcate an area of 1m^2 area in the four corners and centre and diseased onion plants in the marked areas counted and recorded.

Disease incidence was determined by counting the number of both healthy and diseased onion plants within each square meter and the incidence obtained by using the formula below:

$$\text{Incidence (\%)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants within a square meter area}} \text{ X } 100$$

(Wheeler, 1969)

The disease severity was scored following the symptoms on a 1 to 5 (Table 5) severity scale and determined by using the severity index formula (Hicks, 2004);

$$\text{Severity Index} = \frac{\text{Class rating x Class frequency}}{\text{Number of indiduals examined}}$$

Table 5: Disease severity scale for onion bulb rot

Index (%bulb rot)	Qualitative rating	Pictorial rating
1	No symptom on plant (0%)	
2	Curving, yellowing with tip die back (< 25%)	
3	Rot on stem (25 > 50%)	
4	Brown watery appearance on leave base (51 > 75%)	
5	Rotten bulbs (76 > 100%)	

Modified after Lopez, (2003); Jessica *et al.*, (2006).

3.3. Isolation and identification of causal organisms of the bulb rot disease of onion

3.3.1 Preparation of Media

Water Agar media was prepared in a proportion of 3.0 g dehydrated agar to 100 ml of distilled water in a 250 ml conical flask. The conical flask was then plugged with non-absorbent cotton wool and covered with aluminum foil. The mixture was then autoclaved at 1.05 Kg/cm^2 pressure and 121 ^oC for 15 minutes, after which the cotton wool plug was removed, the neck of conical flask flamed and about 10 ml portion of the water agar poured into sterilized 9 cm Petri dishes and allowed to solidify.

Potato Dextrose Agar (PDA) was prepared by dissolving 4 g of PDA powder in 100 ml of distilled water in a 250 ml conical flask. The conical flask together with the content was shaken, covered with aluminum foil and autoclaved at 1.05 Kg/cm^2 pressure and 121 ^oC for 15 minutes. It was then poured (appx 10 ml) in to 9 cm Petri dish and allowed to cool.

3.3.2 Isolation

Isolation of causal agent from the diseased samples was first done on Water Agar (WA) and sub-cultured on Potato Dextrose Agar (PDA). The diseased samples were washed thoroughly under tap water to remove soil and other debris. The samples were then cut opened and tissue segments from advancing margins of diseased tissue removed with flamed scalpel, surfaced sterilized in 1% sodium hypochlorite solution for two minutes and plated on the WA.

The plates were incubated at 26 ± 1 ^{o}C and periodically observed for mycelia growth. Mycelial plugs were cut with a sterilized 2 mm cork borer and transferred to PDA plates under aseptic conditions and incubated at temperature of 26 ± 1^{o}C for 10 days. The method employed for the identification was that of Pathak, (1984) Kishore, (2007) Sara, (2010).

3.3.3. Identification

3.3.3.1 Identification based on cultural charateristics

Identification of the fungi was based on spore morphology and culture characteristics, as described by Booth (1971) and Schwartz (2010) and by its pathogenicity to the onion bulb (Agrios, 2005).

3.3.3.2 Molecular identification

A further confirmation of the species of the causal organism of the bulb rot disease of onion was done by molecular characterization (Vijai *et al.*, 2010) since the technique is specific in identifying a pathogen.

The single spore isolation technique (Vijai *et al.*, 2010) was used to obtain a culture of the fungus from diseased onion plants. The single spore was obtained by preparing spore suspension from an acervulus of the isolated pathogen which was then streaked on water agar and incubated at room temperature for two days. The plate was inverted and observed under a compound microscope (x20). The tip of hypha growing from the single spore was excised and transferred to PDA plates with the aid of a cork borer under aseptic conditions and incubated at temperature of 27±10^{0}C for seven days. The pure cultured isolate was sent to the Biotechnology Centre, University of Ghana for identification with a specific primer.

3.3.3.2.1 DNA extraction

Extraction of DNA from the fungus was carried out using Genomic DNA isolation protocol (Fulton *et al*., 1995). One hundred milligrams of fungal mycelia obtained from a seven-day-old culture were harvested with a sterile toothpick into an autoclaved frozen mortar, ground into a fine powder and placed in the bottom of a 1.5 mL eppendorf tube. Fresh microprep buffer (Appendix 11) was prepared and kept at room temperature. The buffer (750 µ1) was added to the ground mycelia, vortexed lightly and incubated at 65 ^{0}C in a waterbath for 60 minutes. The tube was filled with chloroform/isoamyl (24:1), vortexed and vigorously shaken to mix. The tube was centrifuged at 10,000 rpm for 5 minutes and the aqueous phase pipetted off into new microfuge tube. Cold isopropanol ($^{2}/_{3}$ to 1 times the volume) was added to precipitate the DNA. The tube was immediately spun at 10,000 rpm for 5 minutes and the isopropanol decanted and DNA pellets washed with 70% ethanol. The DNA pellets were air-dried by leaving tube upside down on paper towel for one hour, resuspended in 50 µ1 of TE buffer (10 mM Tris HCI, pH 7.5, 1 mM EDTA) at 65 ^{0}C for 15 minutes and spanned 10 minutes at 10,000 rpm. Extracted DNA was stored at 4 ^{0}C in a refrigerator for one week.

3.3.3.2.2 Polymerase chain reaction (PCR) amplification

A specific oligonucleotide primer for *Fusarium oxysporium* f. sp. *cepae* (OPA -03; AGTCAGCCAC) (Harun, 2010) was used. PCR was performed in Eppendorf Master

Cycler in 25 µl reaction volume containing 25 ng genomic DNA, 0.4 µl (5 pmole) primer, 1.5 µl dNTPs (25 mM), 3 µl of 10 X assay buffer with $MgCl_2$ (15 mM), 0.5µl (3 U/µl) of Taq DNA polymerase (Vijai *et al.,* 2010), distilled de-ionised water was added to make final concentration. The reaction profile was as follows: Initial denaturation for 5 minutes at 94 °C followed by 35 cycles of 1 minute each at 94 °C and 35 °C, followed by 2 minutes at 72 °C, and a final extension for 5 min at 72 °C. PCR (6 µl) amplification was carried out in a BIO-RAD; cyder Thermocycler. PCR products were run on a 1.5% agarose gel stained with 1 µl of 10 mg/mli ethidium bromide.

3.3.3.2.3 Gel electrophoresis

A 1.5% agarose gel was prepared for a 114 cm electrophoresis tank by weighing 1.71g of agarose powder into 114 mL IX TAE buffer in a conical flask. The volume of the solution was weighed after which the agarose mixture was heated in a microwave till the agarose dissolved. The solution was reweighed after heating and water added to make up to the original volume of the solution. Five microliters (5 µL) ethidium bromide was added to the final solution and the conical flask swirled gently to mix the solution. The gel was then poured after cooling into a casting electrophoresis tank with wells set in place and left to set. The combs were removed after 35 minutes and the gel placed in an electrophoresis tank such that wells were oriented at the anode and filled with IX TAE. DNA samples (5 µL) were mixed with loading dye and gently loaded into the wells using a sterile pipette. The electrophoresis tank was closed, electrical leads were then attached to the tank and the DNA samples run at 80 V for 60 minutes after which the gel was removed and visualized using the gel documentation system.

3.4 Confirmation of pathogenicity of isolated fungus

Pathogenicity of the fungal isolate was tested on onion bulbs (Texas Grano) purchased from the Madina market in Accra and also on Malavi cultivar brought from the field. The outermost dry scales of each bulb were removed and surface disinfected in 0.5% NaOCl. for three minutes.

The bulbs were inoculated by removing tissue plugs from onion bulbs using a sterile 5 mm diameter cork borer which was pushed 8 mm deep. The wells created were filled with mycelial plugs removed from the margins of three-day old cultures of the *Fusarium oxysporium* isolated. The plugs were covered with the section of scales that had been removed, and the inoculation site covered with Para film. The control was set up in similar manner but inoculated with a sterile PDA. The bulbs were incubated at room temperature (26 ± 1 ^{o}C) for 30 days.

3.5 Evaluation of fungicides and biological control agents for inhibition of radial growth of mycelia of *Fusarium oxysporium*. f. sp. cepae

Two biological agents (*Trichoderma asperellum*, and *Bacillus subtilis*) and two synthetic fungicides (Carbendazim and mancozeb) were assayed against *Fusarium oxysporium*. f. sp. *cepae in vitro*.

3.5.1 In vitro evaluation of efficacy of biological control agents

The dual culture technique for evaluation of the biological control agents was used (Zlata *et al*., 2008). Mycelia discs of 2 mm diameter of FOC were inoculated at one end of the Petri plate containing sterile PDA and the *T. asperellum* plated 6 cm apart. The *Bacillus subtilis* (bacterial antagonist), was streaked in the middle of the Petri dish (Kishore, 2007) and 2 mm mycelia discs of FOC placed 3 cm apart at the ends. The control plates contained mycelia discs of 2 mm diameter of FOC placed in the middle and each treatment was replicated three times.

The plates were then incubated at 27±1 ^{o}C for ten days and the zone of inhibition recorded by measuring the daily radial growth of mycelium of the test fungus and the antagonistic organism towards the clear zone. The colony diameter of the FOC in control plate was also recorded.

The percentage inhibition of mycelial growth of the FOC was calculated using the following formula:

$$I = \frac{C-T}{T} \text{ X } 100 \qquad \text{Vincent (1947)}$$

Where:
I= Per cent inhibition
C = Radial growth in control
T = Radial growth in treatment

3.5.2 *In vitro evaluation of efficacy of synthetic fungicides against Fusarium oxysporium.* f. sp. Cepae

The efficacies of the two synthetic fungicides (Carbendazim and Mancozeb) were assessed using the manufacturers recommended rates shown in Table 6 below.

Table 6: Fungicides and their formulations

Fungicide	formulation	Recommended rate
Carbendazim	50% WP	50 gms/15L of H_20
Mancozeb	70% WP	75 gm/15L of H_20

Recorded rates of the individual fungicides were added separately into molten PDA and shaken thoroughly to get the desired concentration. Later 20 ml of the poisoned medium was poured into sterile Petri plates. Mycelial discs (2 mm) from actively growing culture of the FOC were cut with a sterile cork borer and placed at the centre of each poisoned agar plate. Control was maintained without adding any fungicides to the medium. Each treatment was replicated three times and the plates were incubated at 28 ^{o}C for ten days. Radial colony growth of the FOC was measured daily for 7 days. The efficacies of the fungicides were expressed as percentage inhibition of mycelia growth over control and calculated by using the formula suggested by Vincent (1947).

Five mycelia plugs of 6 mm diameter from a 10-day-old FOC on PDA were excised, suspended in 20 ml distilled water and shaken thoroughly to obtain uniform spore suspension. A drop of the spore suspension was placed on a haemocytometer and the number of spores counted. The number of spores per ml was then calculated using the formula:

$$\text{No. of spores per ml} = \frac{N}{X} \text{ X } 100 \qquad \text{Pathak (1984).}$$

Where:

N = Total no. of spores counted/ no. of squares
X = Volume of mounting solution between the cover glass and above the squares counted.

3.6 Seed health test to determine the presence/absence of Fusarium oxysporium.**f. sp** *cepae in onion seeds*

The study was conducted to determine if *Fusarium oxysporium* f. sp.*cepae* was seed bore, and also

i. Detect the presence or absence of other external pathogens
ii. Evaluate the germination % of the seeds to be used for the experiment and

Fifty (50) seeds of each cultivar were examined for damage and subsequently soaked in distilled water for two hours after which slides of the spore suspension were prepared and observed under a compound microscope magnification for surface contaminating pathogens.

The seeds were then surfaced sterilized and the seed embryo and endosperm separated with a sterile scalpel under a stereomicroscope. Ten dissected seed embryos and endosperms were placed in ten Petri dishes containing potato dextrose agar (PDA). A set of ten intact unsterilized seeds were soaked in distilled water for 2 hours and a second non-soaked set of the seeds were placed in Petri dishes containing moist filter paper and PDA. Ten Petri dishes containing PDA only were used as controls. All Petri dishes were incubated at 26 ^{o}C for 10 days and colonies of fungi which developed on agar around the samples were identified. All identifications were made based on morphological characteristics of fungi in accordance as described by Barnett and Hunter (1998).

Percent incidence of fungi was recorded using the formula:

$$\text{Percent incidence} = \frac{\text{Number of infested seeds}}{\text{Total number of seed examined}} \times 100$$ (Koike *et al*., 2007)

The seed germination and survival tests were done at the National seed testing laboratory of the Ministry of Food and Agriculture (Plant Protection and Regulatory

Services Directorate, Pokuasi) in Accra using the Jacobson incubator (ISTA 2012). The evaluations were based on the International Rules for Seed Testing (ISTA 2012).

3.7. Screening of onion cultivars for resistance/tolerance to *Fusarium oxysporium* f. sp. *cepae*

This experiment was conducted in two phases involving seedling screening and screening of matured plants. Four onion cultivars namely Bawku Red, Malavi, Texas grano and Red Creole (Table 7) were screened for their reaction (s) to *Fusarium oxysporium* f. sp. *cepae*.

Data recorded were used to draw disease progress curves using the formula below (Shaner and Finney, 1977) and the values statistically analyzed using analysis of variance. Where there were significant differences, least significant difference ($LSD_{5\%}$) was used to separate the means. Genstat software 9.0 versions were used to perform the analysis.

$$\text{AUDPC} = \sum_{i=1}^{n} [(X_{i+1} + X_i)/2]\,[t_{i+1} - t_i]$$

where, X_i = the proportion of the host tissue damaged at i^{th} day

t_i = the time in days after appearance of the disease at i^{th} day

n = the total number of observations

3.7.1 Seedling screening

The seedling screening procedure was adopted from Lopez (2003), using freshly prepared fungal spore solutions from ten days old culture. Fifteen Petri plates (90 mm) containing 10 day old cultures of FOC were mixed in a blender for two minutes, sieved through cheese cloth and suspended in distilled water and shaken thoroughly to get a uniform spore suspension. Haemocytometer was then used for the spore by count which was adjusting to 4.0 X 10^4 spores per ml of distilled water. Onion seeds for the test were surface sterilized with 1% sodium hypochlorite solution for three minutes to remove surface contaminants. Table 7 shows the list of onion cultivars used in the screening.

Table 7: List of onion cultivars used for the screening, the place of origin and the supplier

Common name	Place of origin	Supplier
Bawku Red (local)	Bawku (Manga) Ghana	CSIR/SARI
Malavi (local)	Togo	Farmer
Red Creole	Exotic	Input supplier
Texas grano	Exotic	Input supplier

CSIR/SARI – Council for Scientific and Industrial Research/Savanna Agricultural Research Institute

Soil in this experiment was sterilized in an oven at temperature of 120 ^{o}C overnight and allowed to cool overnight before used (Cramer, 2000).

The experiment was conducted at the SINNA garden of the Crop Science Department (UG). Plastic trays filled with 3 kg of sterilized soil were used. Each tray contained 28 pots or chambers in a 4 x 7 arrangement. A randomized complete block design (RCBD) was used with four treatments, each replicated four times and a control treatment as a check. Each pot in a tray was sown with five seeds at 1.5 cm depth in the inoculated soil and seven pots constituted a replicate. There were four replications for each cultivar. Seedlings of all four cultivars of onion replications were inoculated with *Fusarium oxysporium* f. sp *cepae* isolate except the control treatment. To maintain a homogenous concentration of 4 x 10^4 spores per gram of sand, 2 x 10^8 spores were delivered with 400 ml of distilled water in each tray. The spores were thoroughly mixed in the trays before planting of seeds. Only 400 ml of distilled water was poured in the control trays. Counts of dead and living seeds and seedlings were taken after two and four weeks and the percentage of emergence (survival) calculated after Saxena and Cramer (2009).

$$\% \text{ Emergence} = \frac{\text{Number of seedlings emerge}}{\text{Total number of seeds planted}} \times 100$$

$$\% \text{ survival} = \frac{\text{Number of seedlings survived}}{\text{Total number of seedlings emerged}} \times 100$$

3.7.2 Screening of matured onion cultivars for resistance/tolerance to Fusarium oxysporium **f. sp.** *cepae (pot experiment)*

The cultivars used were Bawku Red, Malavi and Red Creole. Texas grano was not used as it was not viable. Seedlings of these cultivars were transplanted on February 14th, 2012.

A randomized complete block design was used with three replications. Four weeks old seedlings were transplanted in seedling bags (18.5 x 26 cm) filled with 2.3 kg sterilized soil placed in metal seedling trays. The soil in the pots was inoculated with suspension of *Fusarium oxysporium f. sp cepae* isolate ten days before and two weeks after transplanting. The inoculation was done by stirring the soil (8 or 10cm deep) around the seedling in the pot, and a 100 ml of the spore suspension (approximately 10^8 spores per pot) poured into the soil.

The spacing between onion plants in pots was 20 cm and plots within a row were 40 cm apart and 50 cm between rows (Trays/reps) was 50 cm. The onion crop was sown and managed as per the standard practices for Ghana suggested by Kyofa-Boamah *et al*. (2000). Plant-Prod (NPK) 6-11-31(a hydroponic fertilizer) was used in conjunction with Calcium nitrate 15.5-0-0 at the rate of 115 and 85 grams per 100 liters of water respectively. The concentration of plant-prod and calcium nitrate were maintained at the rate of 50 g and 40 g per 100 ml respectively. The product was further dissolved at the rate of 60 ml/litter of water and sprayed on crops at two weeks interval. Cydim super (a synthetic insecticide) was used at the rate of 2.3 ml/ litter of water to control insect. All metal trays and soil used for the experiment were sterilized before disposal after the experiment.

The incidence and severity of the disease were determined weekly for ten weeks after transplanting (WAT), by counting and scoring with the 1 to 5 severity scales (section 3.2): one (Healthy) to five (Rotted bulbs) as indicated in section 3.2 (Table 5). The results were Arc sin transformed using and analyzed using Generalized Linear model of (GLM) SAS 9.1.3 service pack 4 (2004) (Appendix 12).

Onions bulbs were evaluated for disease severity at harvesting and later at four weeks after harvest (Jessica *et al.,* 2006). At maturity all the bulbs of individual plots were harvested and four randomly selected bulbs from each plot were cut transversely through the basal plate. The disease severity was then rated on a scale of 1(no disease) to 5(rotted bulb): **1 (**>15%), **2** (25% diseased), **3** (50%), **4** (75%), **5** (95%) (Modified after Gutierrez and Cramer 2005) The remaining bulbs were kept in storage. Data from the four bulbs from each plot were used to calculate the mean disease severity for that entry and the incidence calculated from percentage of sample bulbs with symptoms of infection by *Fusarium* basal rot. Onion bulbs that rated four and above were included for calculating incidence and severity at harvest, but not stored for the disease progression study (Gutierrez and Cramer, 2005). Bulbs discarded for this reason were replaced with new bulbs that were rated. The same four bulbs were rated after four weeks of storage and disease progression observed on each bulb. Data were subjected to analysis of variance. LSD (5%) was used to separate the means using Genstat software 9.2 versions (2007).

3.8 Evaluation of the efficacy of biological control agents and fungicides against *Fusarium oxysporium* f. sp. *Cepae* (*In vivo*)

This experiment was carried out in a Randomized Complete Block Design with five treatments and three replications with each plot consisting of four plants. Four antagonistic agents in table 8 (Carbendazim, Mancozeb, *Trichoderma asperellum* and *Bacillus subtilis* were used). Distilled water was used as a check.

Table 8: Synthetic and biological fungicides and their mode of action

Name of agent	Group	Mode of action
Carbendazim 50 % WP	Fungicide	Systemic
Mancozeb 75% WP	Fungicide	Protectant
Trichoderma asperellum	Fungus	Biocontrol (Pathogenic)
Bacillus subtilis	Bactria	Biocontrol (Pathogenic)
Distilled water (Contact)	-	Neutral

Malavi, one of the most susceptible onion cultivars from the seedling screening test was used as the experimental material since it is also readily available and affordable to farmers. Four weeks old nursery raised seedlings were transplanted on 22nd February 2012 in potting bags (18.5 x 26 cm) filled 2.3 kg of sterilized soil. The soil in each filled bag was inoculated 10 days before and 14 days after transplanting with a suspension of *Fusarium oxysporium* f. sp. *cepae*. The inoculation was done by mixing the soil with 100 ml approximately10^8 spores per pot of suspension and stirring to a dept of at 8 – 10 cm around the seedling. Each of the antagonistic agents was applied three times to the crop (Rajapakse and Edirimanna, 2002) as follows:

i. Soaking seeds in a suspension of the antagonistic organisms or fungicide for 30 minutes before nursing.

ii. Dipping roots of four weeks old seedlings in a suspension of the antagonistic organisms or fungicide for 30 minutes before transplanting.

iii. Spraying of antagonistic organisms or fungicide at 4 and 6 weeks after transplanting and two weeks before harvest.

The incidence and severity of the disease were determined weekly for ten weeks after transplanting, and was done by counting and scoring with 1-5 severity scales on all the experimental plots. The above ground symptom scoring was based on total top and bulb rotten and ranged from one (Healthy) to five (Rotted bulbs) as indicated in section 3.2.3. Onions bulbs were evaluated at both harvesting and four weeks after harvesting as suggested by Cramer (2000). Maturity of the crop was decided when over 80% of the tops within a plot wiltered. Onions bulbs were evaluated for disease severity as described in section 3.6.2 above.

CHAPTER FOUR

4.0 RESULTS

4.1. Farmers knowledge and perceptions of onion bulb rot disease

The results obtained from the interviews and questionnaire administered to 40 onion farmers each from the Fateakwa and Kwahu south districts on the prevalence, spread, economic impact and control of onion bulb rot disease are presented below.

4.1.1 General background of respondents

Out of the 80 respondents 91.2% were males and 8.8% were females. About 22.5% had no formal education while 77.5% had formal education including Junior High School and the tertiary level (Table 9). Most of the farmers (93.8%) were small-scale peasant farmers with farm size of less than 6 acres. Only 6.2% were large-scale farmers with more than 7 acres.

Table 9: Level of education of onion farmers in Fanteakwa and Kwahu South districts

Level of education	Number of farmers	Percent
Middle School Leaving Certificate	46	57.5
Junior High School	11	13.8
Senior High School	2	2.5
Tertiary Institution	3	3.8
No Formal Education	18	22.5
Total	**80**	**100.0**

4.1.2 Farmers' perception on the prevalence and symptoms of diseases that affect onion in the study area

Majority (60%) of the farmers considered onion bulb rot as the most prevalent onion disease in the area followed by leaf twister (30%) and seedling dumping off (10%), (Table 10).

Table 10: Rating of prevalence of onion diseases by farmers in the Fanteakwa and Kwahu south districts of the Eastern Region

Disease	**% of farmers**
Bulb rot	60.0
Leaf twister	30.0
Dumping off	10.0

Most of the farmers (68%) indicated that bulbs affected by disease produced a foul smell; the rest reported leave dieback and chlorosis (22.5%) or yellowing and twisting of leaves (8.8%).

4.1.3 Farmers' *perception about causes of onion bulb rot and susceptibility of onion cultivars to the disease*

The cultivars of onion planted by farmers the two districts are shown in Table 11.

In 2007, 62.5% of the farmers planted Bawku Red and 37.5% planted Malavi, and in 2008, 47.5% planted Bawku Red and 52.5% planted Malavi. In 2009, 3.8% planted Bawku Red and 96.2% planted Malavi. Whiles 1.2% planted Bawku Red and 98.8% planted Malavi in 2010 (Table 11).

Table 11: Cultivars of onion planted between 2007 and 2010

Cultivar planted	**Percentage of farmers**			
	(2007)	(2008)	(2009)	(2010)
Bawku Red	62.5	47.5	3.8	1.2
Malavi	37.5	52.5	96.2	98.8

Almost all the farms had experienced onion bulb rot disease but most farmers (62.5%) perceived the disease to be caused by continuous rainfall while 37. 5% attributed the disease to soil organisms (Table 12).

Table 12: Farmers perception on causes of onion bulb rot disease at Fanteakwa and Kwahu south districts of Eastern region

Causes	**Frequency**	**% respondents**
Soil organism	30	37.5
Rainfall	50	62.5
Total	80	100

All farmers indicated that the two main cultivars of onion namely Malavi and Bawku Red were both susceptible to onion bulb rot disease. Over fifty percent (53.8%) of the farmers indicated that Bawku Red was the most susceptible while the rest (37.5%) thought Malavi was moderately resistant. Only 8.8% thought the disease equally affected both cultivars.

4.1.4 Perception of farmers on the spread and control of onion basal rot disease

Fifty percent of the farmers indicated that the disease was spread within farms by wind, 27.5% attributed the spread within farms to rainwater whiles 22.5% attributed it to human activity. Majority (72.5%) of farmers reported that the disease was by wind while the rest either attributed it to rainwater (18.8%) or human activity (8.8%).

Seventy percent of the farmers indicated that the disease could be controlled by use of synthetic pesticides, while the rest (10%) felt it could not be controlled. A few of the farmers (5%) were undecided. Some, however, suggested farm sanitation for control of the disease and 15% of the farmer's mentioned crop rotation (Table 13).

Table 13: Methods of control of onion bulb rot by farmers

Method of control	Percentage of farmers
Chemical control	70
Crop rotation	15
Could not be controlled	10
Indifferent	5
Total	100

Majority (over 90%) of the farmers observed the disease incidence to be high during the mid season especially when it rained continuously for more than three days.

4.1.5 Perception of farmers on economic importance of bulb rot disease of onion

The survey was conducted on expected yield and actual yield obtained by farmers for 2009 and 2010. In 2009, none of the farmers expected to get between 1875-265 kg of onion per hectare while 8.8% expected to get at least between 2700-3375 kg/ha. Seventy percent expected 3450-4125 kg/ha while 21% expected 4200-4875 kg/ha at the beginning of the season. However, at the end of the season 45% of the farmers harvested below 2625 kg/ha, 40% of the farmers obtained between 2700 kg/ha, 15% obtained between 3450-4125 kg/ha and none of them had between 4200 to 4875 kg/ha (Table 14).

In the year 2010 a few of the farmers (2.5%) considered themselves among those likely to get less than 2625 kg/ha whereas 73.8% expected to get 2700-3375 kg/ha. 21.2% expected to get 3450-4125 kg/ha and only 2.5% expected to get 4200-4875 kg/ha. At the end of the 2010 season, unfortunately 58.7% harvested less than 2625kg/ha whiles 33.8% harvested between 2700-3375 kg/ha. 7.5% obtained between 3450-4125 kg/ha and none harvested the expected 4200-4875 kg/ha (Table 14).

Table 14: Impact of onion bulb rot disease and other unknown factors on expected and actual yield obtained by farmers in 2009/2010 farming seasons

Yield of onion per hectare (kg)	Expected yield at the beginning of 2009 (% of Farmers)	Actual yield at end of 2009 (% of Farmers)	Expected yield at the beginning of 2010 (% of Farmers)	Actual yield at end of 2010 (% of Farmers)
1875 -2625	0.0	45	2.5	58.7
2700 - 3375	8.8	40	73.8	33.8
3450 - 4125	70	15	21.2	17.5
4200 - 4875	21.2	0.0	2.5	0.0

A bag of onion weights 75 kg

4.1.6 Incidence and severity of onion bulb rot disease in the Fanteakwa and Kwahu south districts

The onion bulb rot disease incidence of 23.24% at Manguasi was significantly higher ($p < 0.05$) compared to Abuakwa and Mpaem Quarters which had 1.8 severity index. Abuakwa had 17.10% incidence and severity index of 1.5. Mpaem Quarters recorded the lowest incidence of 10.18% and severity of 1.2 (Table 15, Appendix 2).

Table 15: Incidence and severity of onion bulb rot disease in the communities surveyed in Kwahu south and Fanteakwa districts

Community	% Disease incidence	Disease severity
Manguasi	23.24%	1.8
Abuakwa	17.10%	1.5
Mpaem Quarters	10.8%	1.2

Disease incidence was calculated using the formula of Wheeler, 1969 and the severity calculated using the formula given by Hicks, 2004

Among the two districts, the incidence of the disease was higher in the Kwahu south district at 25.3% and severity of 1.5 but lower in the Fanteakwa district with incidence of 17.1% and severity of 0.6 (Table 16). However, there was no significant difference ($p < 0.05$) in the disease incidence but difference in disease severity was significant ($p < 0.05$) between the two districts (Appendix 3 and 4).

Table 16: Incidence and severity of onion bulb rot disease in the Kwahu south and Fanteakwa districts

District	Disease incidence (%)	Severity index
Kwahu South	25.3%	1.5
Fanteakwa	17.1%	0.6

Disease incidence was calculated using the formula of Wheeler, 1969 and the severity calculated using the formula given by Hicks, 2004.

4.2 Microorganism associated with bulb rot disease of onion

The fungus consistently isolated from diseased onion samples from nurseries and farms in the Fanteakwa and Kwahu South districts produced pinkish cottony mycelium with an irregular margin at maximum growth and filled a 9 cm Petri dish in 7 days at 22 to 30 ^{o}C and 65 to 70% RH (Plate 2).

Plate 2: A seven-day culture of *Fusarium oxysporium* on Potato Dextrose Agar

Macroconidia were uniform in size with dorsa ventral curvature along the entire spore and had not less than three septations which measured 20.5 – 29.5 x 3.0 – 5.0 µm (Plate 3). The apical cells appeared tapered and curved with a slight hook and a pointed base. Micro conidia were oval, elliptical or spherical and spindle shaped without septation and measured 4.0 - 6.0 x 2.0 – 3.5 µm (Plate 4). Chlamydospores were usually vacuolated and spherical and measured 4.5 – 9.0 µm in diameter.

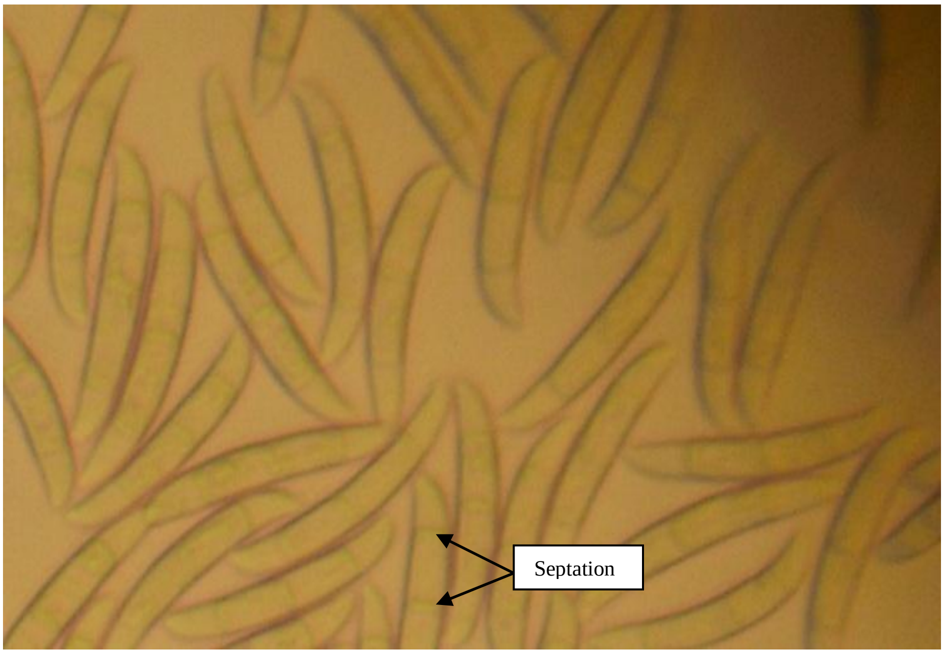

Plate 3: Macroconidia of *Fusarium oxysporium* f. sp. *cepae* isolated from partially rotten onion bulb

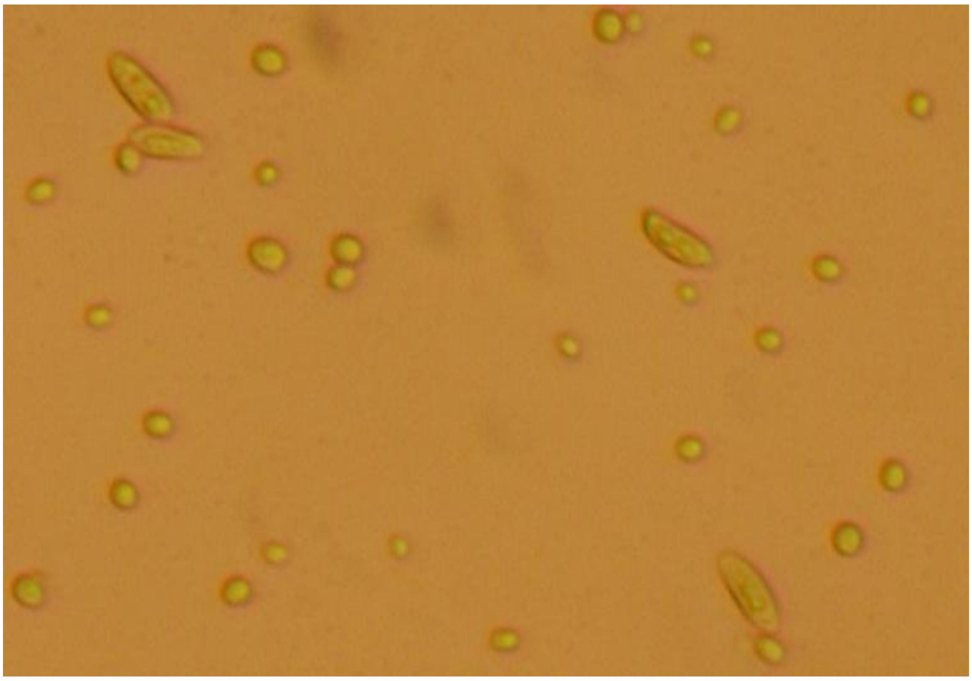

Plate 4: Microconidia of *Fusarium oxysporium* f. sp. *cepae* isolated from partially rotten onion bulb

4.3 Molecular characterization *Fusarium oxysporium* f. sp. *Cepae*

The result of the molecular analysis showed that, the species-specific primer OPA-03 amplified approximately 564 bp fragments from genomic DNA of onion isolate. No PCR product was produced with water control in the reaction (Plate 5).

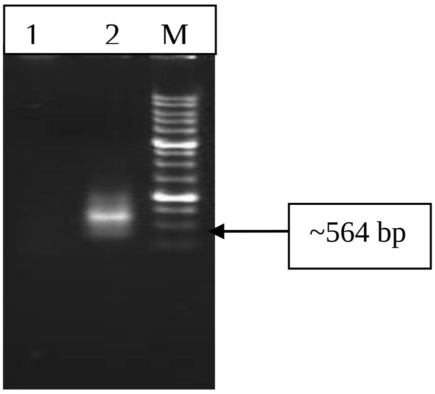

Plate 5: Amplification of specific DNA fragment of Fusarium isolate from onion using an oligonucleotide primer (OPA -03) specific for *Fusarium oxysporium* f. sp. *cepae*, M = molecular size marker GeneRulerTM DNA Ladder Mix (100 to 10,000 bp), 1 = Negative control (water in place of DNA), 2 = Onion isolate.

4.4 Pathogenicity of fungal isolate on inoculated onion bulbs

Symptoms including softening change of colour, sprouting and foul smell were observed on the onion bulbs 28 days after inoculation. Similar symptoms were observed on 90 onion bulbs artificially inoculated.

Fifty eight percent (58%) of the Malavi bulbs rotted completely (>80%) whiles 25% rotted partially (<50%) and 16.7% sprouted without rot. Seventy five percent (75%) of the Texas Grano bulbs rotted while 25% were partial rotten none sprouted. The controls however, remained unaffected by the inoculum (Plate 6). *Fusarium* was subsequently re-isolated from rotted onion bulbs and identified as *Fusarium oxysporium* f. sp. *cepae*.

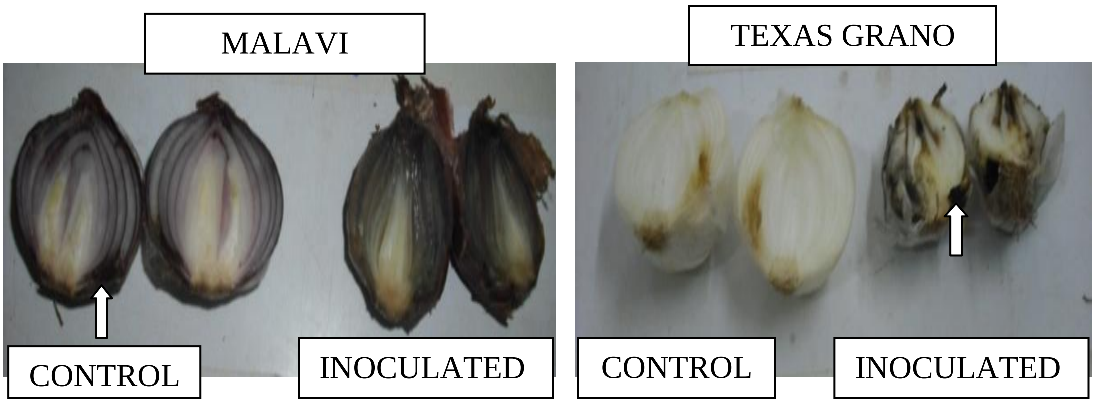

Plate 6: External and internal symptoms of onion bulbs artificially inoculated and uninnoculated with *Fusarium oxysporium* f. sp. *cepae*. Arrow shows point of inoculation

4.5. Effects of Biocontrol agents and Fungicides on radial growth of mycilium and sporulation of *Fusarium oxysporium* f. sp. *cepae (in-vitro)*

Results of the study on the effect of four antagonistic agents (*Trichodema asperellum*, *Bacillus subtilis*, Carbendazim and Mancozeb) on radial growth and sporulation of *Fusarium oxysporium* f. sp. *cepae* (FOC) showed that the antagonistic agents inhibited mycelial growth and sporulation of the fungus either by growing around the FOC or exhibiting an inhibition zone (0.6mm) or by completely reducing growth of the fungus after seven days of incubation.

Of the four antagonistic agents, the two synthetic fungicides (Carbendazim and Mancozeb) provided total inhibition of mycelial growth of the fungus, followed by *Trichodema asperellum* (fungus). *Bacillus subtilis* was the least effective and provided only a zone of inhibition.

Trichodema asperellum was found to be more effective because grew fast and filled the plate (7.5 mm) within seven days of incubation. *Bacillus subtilis* exhibited inhibition zone of 0.6 mm (Plate 7)

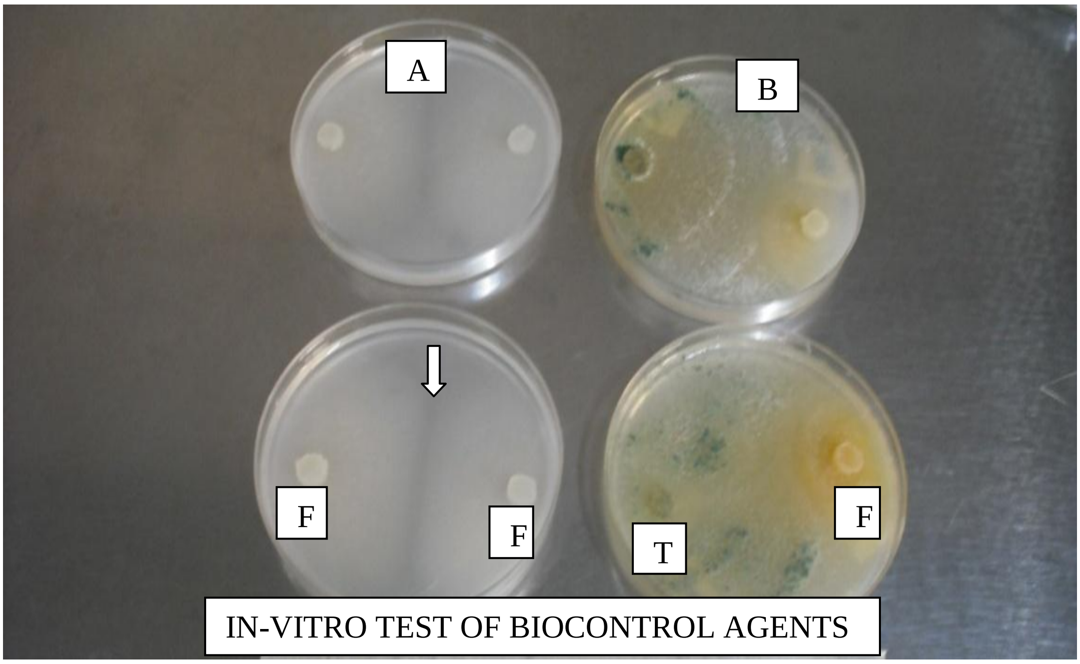

Plate 7: Seven days old culture of *Bacillus subtilis* and *F. oxysporium* (A), and that of *Trichodema asperellum* and *Fusarium oxysporium* (B) in dual culture experiments. Note the Zone of inhibition (*arrow*) and T. asperellum (T) grew over *Fusarium* (F)

The two synthetic fungicides were both effective in inhibiting mycelial growth of *Fusarium oxysporium* f. sp. *cepae*. Mancozeb and Carbendazim were both highly effective at the manufacturer's concentration and gave 100% inhibition of mycelia growth of the fungus. The results also indicated that the rate of mycelial growth inhibition ranged from 20 % - 100 % at seven days of incubation (Table 17).

Table 17: Percentage inhibition of mycelia growth of *Fusarium oxysporium f. sp cepae* by antagonistic agents in bioassay

Antagonistic agent	%Inhibition	Nature of inhibition
Bacillus subtilis	20.00	Inhibition zone
Carbendazim	100.00	Complete inhibition
Mancozeb	100.00	Complete inhibition
Trichodema asperellum	76.50	Over grow FOC

The results also confirmed significant differences ($p < 0.05$) in mycelial growth inhibition among the biological agents. The *Trichodema asperellum* was more effective in mycelia growth inhibition than the *Bacillus subtilis* and resulted in 76.5% inhibition whiles the *Bacillus subtilis* resulted in 20% inhibition. Significant differences ($p < 0.05$) in mycelial growth inhibition were also observed among the biological agents and synthetic chemicals. The synthetic chemicals provided 100% inhibition while the biological agents *B. subtilis* and *T. asperellum* provided 20 and 76.5% inhibition respectively (Appendix 5), percentage growth inhibitions were significantly different ($p < 0.05$) and the mean percentage inhibition ranged from 44.26% to 63.08% at seven days of incubation.

The antagonistic effect on mycelial growth of *F. oxysporium* by *T. asperellum* began from the 3rd to 7th day of incubation with inhibition effects of 1.63% per day (Fig. 1). Growth of the bacterium *B. subtilis* in first two days declined as compared to *T. asperellum*. The antagonistic effect on growth of *F. oxysporium* by *B. subtilis* began from the 5th, to 7th day after incubation. The mycelia of *F. oxysporium* were prevented from growing beyond the inhibition zone by *B. subtilis* from the 4th day of incubation. The two synthetic chemicals Carbendazim and Mancozeb completely inhibited growth (100%) of FOC.

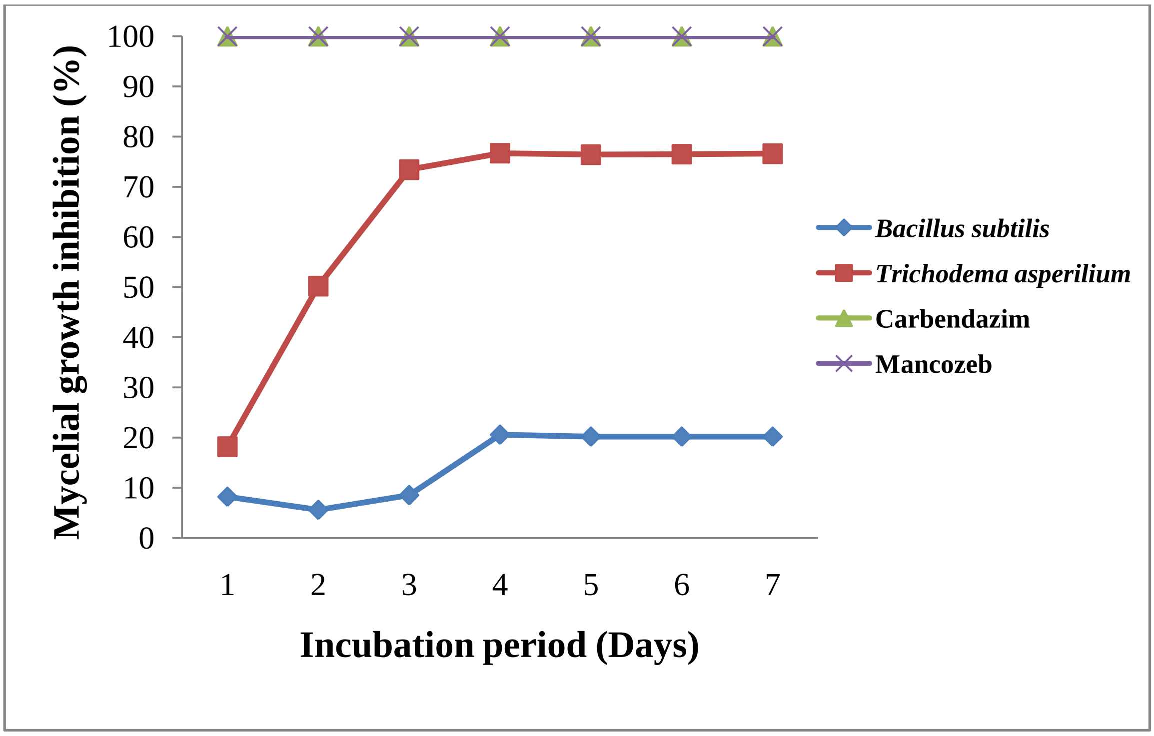

Fig. 1: Mycelial growth rate of *Fusarium oxysporium* f. sp *cepae* by antagonistic agents in bioassay. Note the rapid percentage growth of *T. asperellum* in the first 3 days and decline in the first 2 days of *B subtilis*.

4.6. Microorganisms associated with onion seeds and germination percentage of the seeds

Results of the seed health test conducted on four onion cultivars namely Bawku Red, Texas Grano, Malavi and Red Creole are shown in Table 18 and 19.

Two species of fungi namely *Aspergillus niger* and *Rhizopus stolonifer* were isolated from the onion seeds. The seed coat had the highest contaminant of 80%, followed by endosperm 40% and embryo 10%. The *Aspergillus niger* however, was more prevalent. *Fusarium oxysporium* f. sp. *cepae* the causal organism of onion bulb rot was not isolated from any of the seed parts.

Table 18: Incidence of fungi detected in parts of onion seed

Seed part	% contamination of seed parts		Total
	Aspergillus niger	*Rhizopus stolonifer*	
Embryo	10	0	10
Endosperm	30	10	40
Seed coat	60	20	80

Disease incidence of fungi (%) was calculated using the formula of Wheeler, 1969

Table 19: Percentage germination of seeds tested at the National seed testing laboratory Pokuase (means of four replicates; 50 seeds planted per replicate)

Cultivar	%Germination
Bawku Red	90.5[a]
Malavi	89.0[a]
Red Creole	88.0[a]
Texas	89.5[a]

Means within a column followed by the same superscript are not significantly different at lsd ($p < 0.05$).

4.7. Effects of *Fusarium oxysporium* f. sp. *cepae* on germination of onion seeds and survival of onion seedlings in inoculated soil

Results of the seedling screening conducted in inoculated soil on four onion cultivars (Bawku Red, Malavi, Red Creole and Texas Grano) are shown in Plate 8 and Table 22.

There were significant differences ($p < 0.05$) in emergence and survival among the onion cultivars. The Bawku Red had the highest seedling emergence and survival rates of 72.15% and 65.72% respectively, followed by the Red Creole with seedling emergence rate of 54.29% and survival rate of 42.86%. Malavi had an emergence rate of 45.71% and survival rate of 34.82% whiles Texas Grano had the lowest seedling emergence and survival rate of 33.57% and 25.71% respectively (Plate 8). Seedlings with higher emergence rate also had higher survival rate.

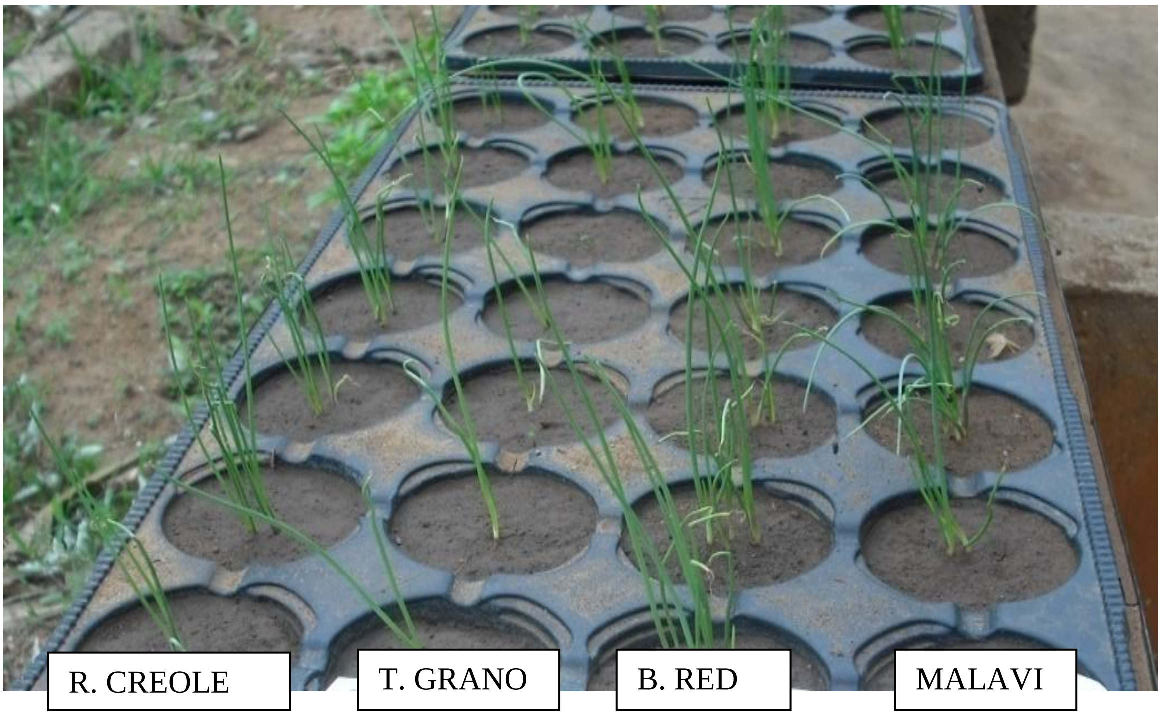

Plate 8: Seedling survival of four onion cultivars (Malavi, Bawku Red, Texas Grano and Red Creole) in soil inoculated with *Fusarium oxysporium* f. sp. *cepae.* **(Each pot in the tray was sown with five seeds and seven pots constituted a replicate. There were four replications for each cultivar)**

Results also indicated that significant differences ($p < 0.05$) existed among the cultivar mean (Appendix 7). However, there was no significantly higher emergence ($p < 0.05$) among the cultivars in the control treatments (Table 20). There was no significant difference ($p < 0.05$) between Malavi and Red Creole with the rest being significantly different ($p < 0.05$) (Table 21).

Table 20: Mean percentage of seedling emergence of four onion cultivars planted in soil inoculated with *Fusarium oxysporium* f. sp. *cepae*

Cultivar	Inoculated soil	Uninoculated (control)
Bawku Red (%)	72.15^{a}	90.00^{a}
Malavi (%)	45.71^{c}	87.85^{a}
Red Creole (%)	54.29^{b}	86.43^{a}
Texas Grano (%)	33.57^{d}	87.14^{a}

Means within columns followed by same superscripts are not significantly different at LSD ($p < 0.05$).

Table 21: Mean percentage seedling survival (4 weeks after transplanting) of four onion cultivars sown in soil inoculated with *Fusarium oxysporium* f. sp. *cepae*

Cultivar	Inoculated	Uninoculated (control)
Bawku Red (%)	65.72^{a}	87.86^{a}
Malavi (%)	34.82^{c}	87.14^{a}
Red Creole	42.86^{b}	85.71^{a}
Texas Grano (%)	25.70^{d}	84.28^{a}

Means within a column followed by the same superscript are not significantly different at ($p < 0.05$)

4.8. Bulb formation of onion cultivars (Bawku Red, Rred Creole and Malavi) sown in soil inoculated with *Fusarium oxysporium* f *sp. cepae*

The results obtained from screening the three onion cultivars are shown in table 22 Plate 9 and 10.

There were significant differences ($p < 0.05$) in the size of bulbs formed from the three onion cultivars inoculated with *Fusarium oxysporium* f.sp. *cepae.* The Bawku Red was the most affected cultivar (Plate 9) and also produced smaller bulbs (Plate 10). In general, all the inoculated plants of the same cultivar produced smaller bulbs compared to their control treatments. They both however, maintained their natural shape and colour.

Statistically, the least affected cultivar was Malavi with disease incidence of 54.15%, severity index of 1.57 and mean bulb weight of 41.62g, followed by Red Creole with incidence of 56.16%, disease severity index of 1.67 and bulb weight of 35.05 g. The most affected was Bawku Red which produced smaller bulbs as compared to the others with incidence of 79.1%, disease severity index of 2.55 and mean bulb weight of 28.03 (Table 22). The control treatments remained unaffected.

The disease progress was significantly different ($p < 0.05$) among the three tested cultivars (Appendix 9). The Bawku Red had the highest area under the disease progress curve (AUDPC) 153.4 followed by Red Creole (103.5) and Malavi (95.1). Cultivars with higher incidence also had higher severity and higher AUDPC.

Plate 9: Effects of *Fusarium oxysporium* f. sp. *cepae* on three onion cultivars planted in inoculated soil. Note B. Red (Curving, leaf die back and Rot on stem) most affected and Malavi least affected

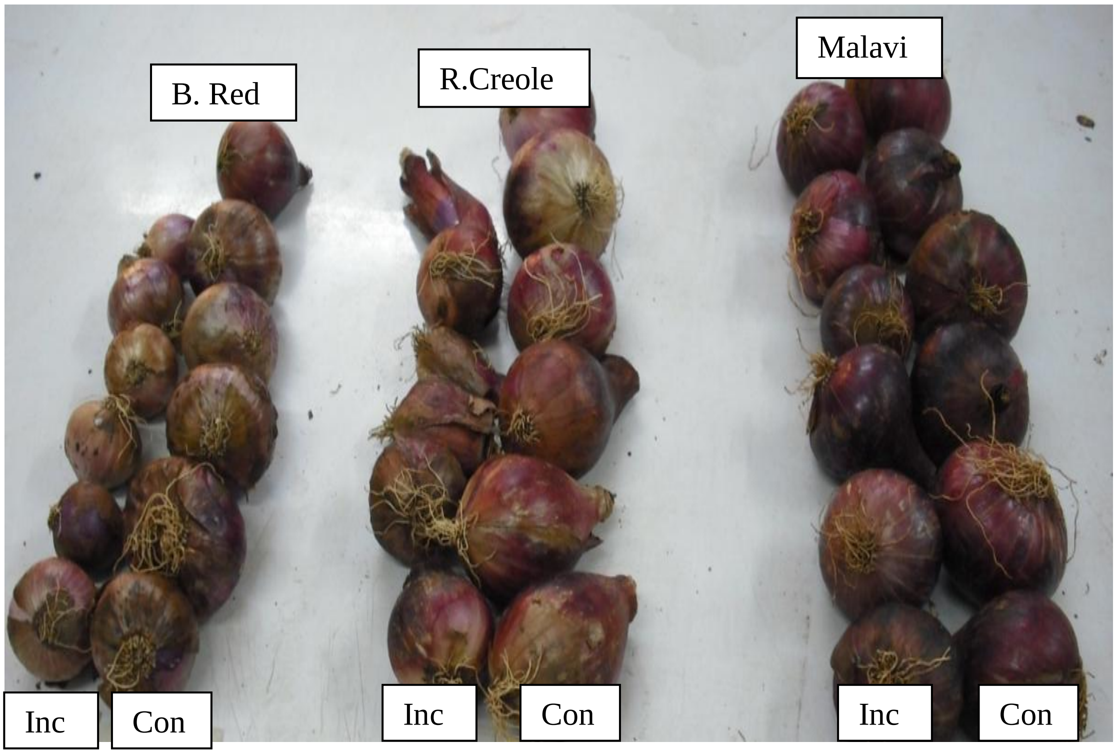

Plate 10: Bulbs produced by three onion cultivars sown in soil inoculated (Inc) and uninoculated (Con) with *Fusarium oxysporium* f. sp. *Cepae* (Note differences in size)

Table 22: Mean incidence and bulb weight of three onion cultivars planted in soil inoculated with *Fusarium oxysporium* f. sp. *cepae*

Cultivar	Incidence (%)	Mean bulb weight (grams)
Bawku Red	79.1	28.03[c]
Malavi	54.15	41.62[a]
Red Creole	56.16	35.05[b]

Means within a column followed by the same letter are not significantly different at LSD ($p < 0.05$)

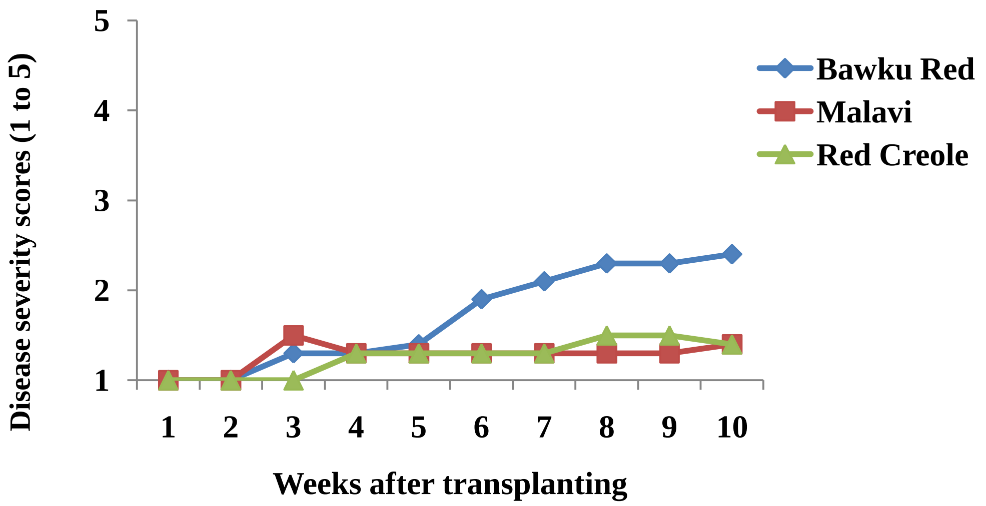

Fig. 2: Severity of onion bulb rot disease on three cultivars of onion planted in soil inoculated with *Fusarium oxysporium* f.sp. *cepae*

4.9 Pot experiment on the effects of Carbendazim, Mancozeb, *Trichodema asperellum* and *Bacillus subtilis* on *Fusarium* bulb rot of onion

When Malavi, a susceptible variety from the seedling screening was treated with the two fungicides and two bio-control agents in inoculated soil against *Fusarium oxysporium* f. sp *cepae* the results obtained are illustrated in Table 23, Fig. 3 and Plate 11. The results revealed significant differences ($p < 0.05$) among the various treatments applied. Carbendazim was most effective with a disease incidence of 3.3% compared to the control (1.0). Mancozeb followed as next best with incidence of 8.9% and a severity of 1.1 followed by *Trichodema asperellum* with incidence of 16.7% and severity of 1.2. *Bacillus subtilis* was found to be least effective with disease incidence of 22.2% and severity of 1.2. The control had 81.1% disease incidence and severity of 2.1 (Table 23).

Control agents that had higher disease incidence also had higher disease severity. The difference in bulb size and weight was not significant ($p < 0.05$) among the treatments. Onion plants treated with Carbendazim produced much bigger bulbs as compared to the control which produced smaller bulbs in size and weight (Table 23, Plate 11).

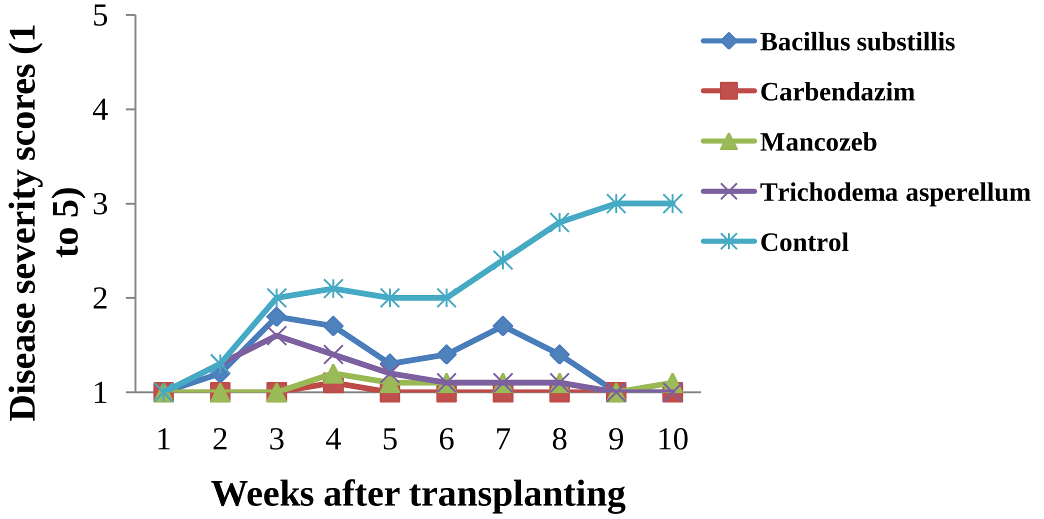

Fig. 3: Progress of onion bulb rot disease on Malavi onion cultivar following treatment with fungicides or biological control agents for management of the disease

Table 23: Mean Incidence and bulb weight following treatment of Malavi (onion cultivar) with Carbendazim, Mancozeb, *Trichodema asperellum* and *Bacillus subtilis* for the control of onion bulb rot disease (pot experiment)

Antagonistic agent	Disease Incidence (%)	Bulb Weight (Grams)
Bacillus	22.2%	45.6^{a}
Carbendazim	3.3	47.6^{a}
Mancozeb	8.9	43.9^{a}
Trichodema	16.7	45.4^{a}
Control	81.1	26.4^{b}

Means within a column followed by the same letter are not significantly different at LSD ($p < 0.05$)

In reference to the performance of the antagonistic agents, the leaves of plants treated with *Trichodema asperellum* changed colour from the normal green to yellowish brown a week after transplanting and regained its normal colour at by the 4th week. Bulbs were round and dark purple at harverst (Plate 11).

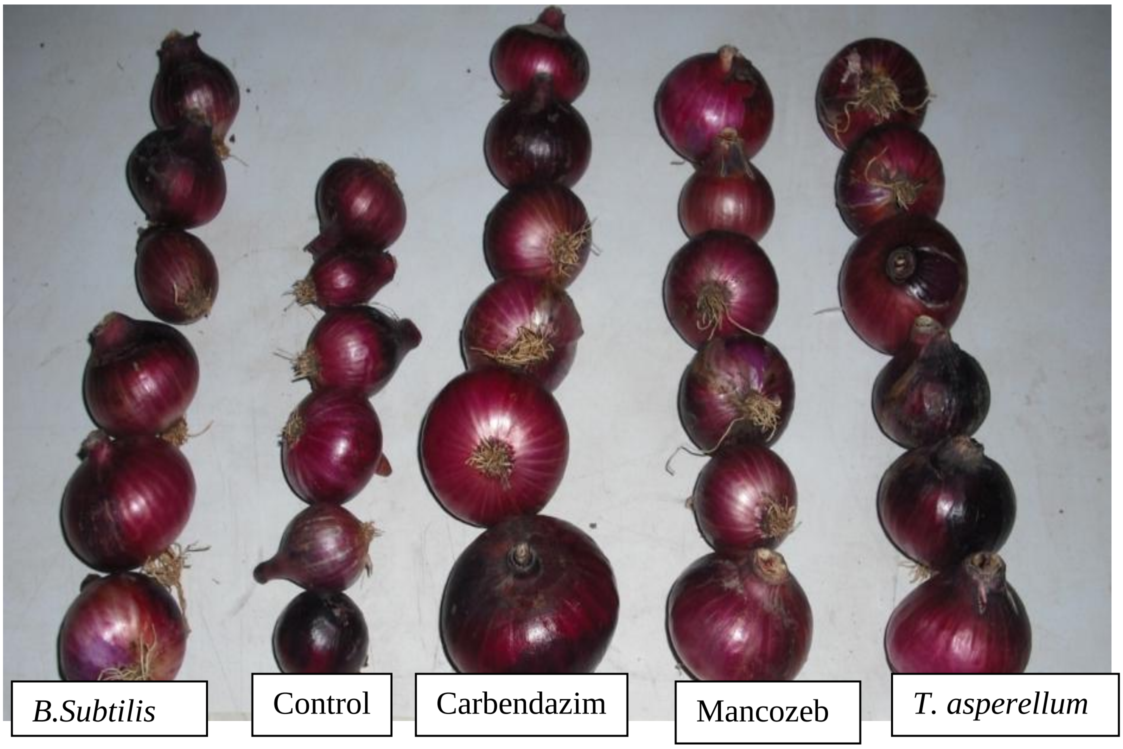

Plate 11: Onion plants (Malavi cultivar) inoculated with FOC. Bulbs of the onion plants treated were bigger than the untreated control. The colour and shape of onion bulbs remains unchanged.

CHAPTER FIVE

5.0 DISCUSSION

Majority of onion farmers in the Fanteakwa and Kwahu south districts were male, women did not show much interest in onion farming because of the risks such as time, soil fertility and rainfall partten. Other reasons given included labour and domestic responsibilities that combined to prevented women from getting much involved in onion farming. One farmer out of the 80 respondents was an adolescent. Education, rural urban drift of the youth and the traditional norm that the youth depends on the family head and work on his farm, were some of the reasons given for the absence of the youthful onion farmers. However, there was an increasing number of commercial onion farms cultivation especially around the Fanteakwa and Kwahu south districts in the Eastern region of Ghana (Awuah *et al.*, 2009).

Diseases that affected onion in the study area included onion bulb rot, onion leaf twister and seedling damping off. According to the respondents, bulb rot disease was the most important production constraint after onion leaf twister. All 15 farms surveyed in this study were infected by the disease and the farmers could identify the disease by its symptoms in the field and had named it 'apropro' after its main symptom which is soft rot of the bulbs (Awuah *et al*., 2009). During the survey it was realized that in attempt to control the disease the onion farmers misapplied pesticides, which had not been effective in reducing the incidence of the disease. A few of them however, used cultural practices such as farm sanitation, weeding and crop rotation. In addition, bulb rot disease of onion could also be controlled through various strategies including use of host plant resistance, solarisation, biological control, integrated pest management and fungicide application (Cramer, 2000; Walker *et al*., 2009; Offei *et al.*, 2008). Losses to *Fusarium* blub rot of onion (FBR) could be significantly reduced through the use of FBR-resistant cultivars (Cramer, 2000). The two main cultivars of onion planted in the area were the red types Malavi and Bawku Red probably because the red cultivars were more tolerant to the disease than the white and yellow cultivars (Apaza and Mattos, 2000; Jessica *et al.*, 2006; Galván, 2009). The interviews revealed that farmers had experienced the problem of onion bulb rot disease in the past years, and had shifted from planting Bawku Red to Malavi. However, Malavi was also found to be susceptible to the disease.

The farmers had little knowledge about the causes of FBR disease and attributed it to continuous rainfall which led to the appearance of the disease after three days. They also ascribed the spread of the disease within and among farms to wind. These perceptions were partially valid. *Fusarium oxysporium* f. sp. *cepae* a soil borne fungus is mostly spread by contact which is enhanced through transportation and planting materials (Agrios, 2005; Howard *et al.*, 2007). Incidence of the disease was highest during the mid season especially when it rained continuously for more than three days since such environmental conditions favored the growth of FOC (Saremi and Bugress, 2000; Havey, 1995). .

During the survey, it was observed that infected onion debris produced after harvesting were left on the field. This practice retains the inoculums on the already mono cultured field (Awuah *et al*., 2009) and results in the persistence of onion bulb rot disease (Cramer, 2000). The incidence and severity of the onion bulb rot disease was different among the two districts and were significantly higher in the Kwahu south than the Fanteakwa district. Gutierrez and Cramer (2005) had similar results of disease differences within the same geographical area. The disease effectively reduced their income and similar result happened in 2010.

The Isolation, pathogenicity tests and PCR analysis carried out in the study confirmed *Fusarium oxysporium* f. *sp cepae* as the causal agent of onion bulb rot disease in Fanteakwa and Kwahu south districts. This is consistent with results reported by Somkuwar *et al.* (1996), Stadnik and Dhingra (1996), Cramer (2000), Awuah *et al.* (2009) and Sara (2010) who isolated the same fungus from rotten onion bulbs in the field in India, Brazil, New Mexico, Ghana and Sweden respectively. Harun, (2010) and Vijai *et al.* (2010) had similar genomic results producing size 564-566bp with OPA-03.

Regarding the four antagonistic agents assayed against *Fusarium oxysporium* f. sp. *cepae*, the two synthetic agents provided complete inhibition of mycelial growth of the fungus followed by *Trichodema asperellum*, which caused 76.5% inhibition after six days of incubation. *Bacillus subtilis* was the least effective and provided only a zone of inhibition of 0.6mm. Monte (2001) reported that a strain of *Trichodema asperellum* was able to overgrow the isolates of *F. solani* mycelia and produce conidia on their surface, indicating mycoparasitic action. Zlata *et al.* (2008) had similar results and attributed the inhibitory action of *T. asperellum* over *Fusarium oxysporium f. sp cepae* mycelia to mycoparasitic action. The results also agreed with Kishore (2007) who reported greater mycelial inhibition of FOC by *Trichoderma* compared to bacterial antagonists and attributed this to higher competitive ability of *Trichoderma* spp. by different mechanisms including mycoparasitism, antibiosis and siderophore production. Sunita and Katoch (2001) had similar results, which agreed with Kulkarni's (2006) report of maximum inhibition of FOC by Carbendazim and Mancozeb. Seeds of the various cultivars used by farmers were tested to determine their health and germination status and to acertain if *Fusarium oxysporium* f. sp. *cepae* was seed borne. Two species of fungi, *Aspergillus niger* and *Aspergillus flavus* were isolated from all parts of onion seed samples especially in the seed coat indicating that *Fusarium oxysporium* f. sp. *cepae* is not seed borne. This result conforms to the findings of Koycu and Ozer (1997).

Results of seedling screening of onion cultivars for resistance to *Fusarium oxysporium* f. sp. *cepae* revealed that all four cultivars of onion were susceptible to the disease. Bawku Red was least susceptible while Malavi and Red Creole were moderately susceptible and Texas Grano was highly susceptible. When Bawku Red, Malavi and Red Creole were used in the mature plant test against the onion bulb rot disease. Malavi was least susceptible while Red Creole was moderately susceptible and Bawku Red was highly susceptible to the onion bulb rot disease and produced smaller bulbs (Awuah *et al*., 2009). Lacy and Roberts (1982) suggested that *Fusarium* basal-rot not only reduces the yield of plants, but also reduces the size of bulbs produced by affected plants. In a similar experiment NuMex Luna' and NMSU 00-32 varieties had the highest seedling survival rate and when the same onion lines were grown in a field where *Fusarium* basal rot (FBR) was endemic, 'Cardinal was found to have the highest FBR incidence whiles NMSU 00-13-1 had the lowest incidence (Lopez and Cramer, 2004). Individual onion lines of the same cultivar varied in their response to inoculation at different crop developmental phases (seedlings, older plants and at mature bulbs) similar observations made by Ozer *et al*.,

1998. Badino *et al*., (1980) also confirmed that the age of seedling also affected resistance levels of onion genotypes.

Carbendazim treatment was found to be the most effective fungicide for the control of FOC in the current study. Even among seven fungicides and biocontrol agents (Kishore, 2007). Carbendazim 50% WP applied at the rate of 30g/10L against onion bulb rot disease decreased field and storage losses of onion bulbs by 35 and 40 percent respectively Rajapakse and Edimanna (2002). Similar results were observed under field conditions, Carbendazim reduced *Fusarium* wilt incidence by up to 80 per cent Sunita and Katoch (2001), while *Trichodema asperellum* was moderately effective. In a similar study *Bacillus subtilis* was the least effective against FOC (Zlata *et al.*, 2008).

Crop rotation and the use of resistant cultivars provide the most cost effective solution to *Fusarium oxysporium* f. sp *cepae* infection leading to onion bulb rot. The widespread availability in soil and distinctive perpetuation mode of the fungus through chlamydospores on crop residues in soil makes the usage of synthetic fungicides impractical or economically although technically capable to control the fungus (Jessica *et.al.*, 2006). Synthetic Chemical measures in management of soil borne pathogens, especially those infecting onion in late vegetative phase, are of limited or no value (Zlata *et al.*, 2008). The presence of a root colonizing antagonist (Biological agents), therefore seems acceptable for many reasons (Monte, 2001), including self-propagation, longer persistence in soil, environment, ecological and toxicological benefit for farmers and consumers (Cosskuntuna and Ozer, 2008). Its disadvantages, however, include short shelf life, sensitivity to environmental effects, and necessity for knowhow education of farmers (Zlata *et al.*, 2008). Managing the health of onion is the focus of many investigations (Cramer, 2002).

Since there is no known onion cultivar bred for resistance to onion bulb rot disease worldwide (Saxena and Cramer, 2009); breeding for disease resistance should be intensified in the country to obtain onion cultivar (s) that can be grown with fewer losses to *Fusarium* bulb rot. There is the need therefore to educate onion farmers of Fanteakwa and Kwahu south districts on Good Agricultural Practices since some of their activities such as poor harvesting, packaging and handling practices contributed to the spread of most onion diseases. The fungal pathogens that cause bulb rot diseases can build up inoculums in soils that are continually planted with onions (Awuah *et al*. 2009). The farmers could also practice crop rotation in a four-year schedule or longer. An effective management program based on Integrated Pest Management could be scheduled for the onion farmers of the Fanteakwa and Kwahu south districts for only two years. This could help reduce the use of fungicides with its accompanying negative effects on the users and the environment.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

- The research revealed that, onion bulb rot disease is widespread in all the onion growing areas in the Eastern region of Ghana. Most of the onion farmers in the Fanteakwa and the Kwahu south districts could identify the disease by its symptoms but had no knowledge of the causal agent and therefore were not controlling the disease. The disease impacted negatively on the livelihood of the farmers.

- The incidence of the disease in onion farms was similar in the Kwahu south (25.3%) and the Fanteakwa (17.1%) districts. The severity of the disease was however, higher in Kwahu south district (1.5) compared to Fanteakwa district (0.6).

- The causal agent of the onion bulb rot disease in the Eastern region of Ghana is *Fusarium oxysporium* f. sp. *Cepae*. *Fusarium oxysporium* f. sp. *cepae* is not a seed born fungus.

- Four cultivars of onion (Malavi, Bawku Red, Red Creole and Texas Grano) were susceptible to the disease. Malavi was least susceptible while Red Creole was moderately susceptible, Texas Grano and Bawku Red were highly susceptible to the onion bulb rot disease, Bawku Red produced smaller bulbs.

- It was also revealed that Carbendazim treatment was most effective straegy for managing onion bulb rot disease in Malavi, Red Creole and Bawku red which produced bigger bulbs compared to the other treatments (Mancozeb, *T. Asperellum and B. Subtilis*)

It is, therefore, recommended that:

- For fields known to produce high incidence of FBR, Malavi could be grown with less losses to *Fusarium* bulb rot than other susceptible cultivars.
- Carbendazim or Mancozeb could be used to reduce the incidence of FBR disease and for long term management the use of *Trichodema asperellum* recommended.
- There is the need to educate farmers on Good Agricultural Practices (GAP) since some of their activities contribute heavily to the spread of the bulb rot disease.
- Farmers are also advised to practice IPM which includes combination of resistant cultivars, cultural control and judicious use of chemical control. More research work is needed on *Trichodema asperellum* on *Fusarium* basal rot of onion.

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APPENDICES

APPENDIX 1: Questionnaire

Survey of farmer's knowledge, perception and experiences concerning prevalence, spread, control, and economic importance of onion bulb rot disease

Questionnaire No………

A. Background

1. Name of farmer……………………………………………….Male/female
2. Level of education…………………………………………………………
3. Name of District……………………………………………………………
4. Name of village……………………………………………………………
5. For how long have you been farming onion …………………………………
6. Size of onion farms, year of cultivation, sources of planting materials, and expenditure……………………………………………………………………

Year	Size	Varieties	Source of planting materials	Expenditure			
				land	labour	Planting materials	others
2007							
2008							
2009							
2010							

B. Farmers Knowledge, perception, and experiences concerning prevalence.

7. What type of diseases affects onion in your area?

………………………………………………………………………………………

8. How does onion look like (from above ground) when they are affected by bulb rot disease?

………………………………………………………………………………………

9. What times of the year do onion plants suffer from bulb rot disease?

………………………………………………………………………………………

10. Which varieties of onions are usually affected by the disease (starts with the least susceptible and ends with the most susceptible variety)?

………………………………………………………………………………

11. Can you describe the stages of the disease? (From above ground)

………………………………………………………………………………………

12. What do you think are the causes of onion bulb rot disease?

…………………..………………………………………………………………………

13. Do you always see bulb rot and other disease symptoms together on a diseased onion plant?

………………………………………………………………………………………

14. If yes which of the two symptoms is observed first?

………………………………………………………………………………………

15. What percentage of the onion farms in your village do you think are affected by the disease?

A) less than 25%, B)between 25% and 50%, C)between 50% and 75% , D) between 75% and 100%, E)100%

C. Knowledge, perception and experiences concerning spread

Which year and month was the disease first heard of?

Year………………………………………Month………………………

16. What do you think is the cause of the disease?

………………………………………………………………………………….

17. What happened before the onion first suffered from the disease?

…………………………………………………………………………………

18. How does the onion disease spread on the same farm?

…………………………………………………………………………………

19. How does the disease spread from one farm to another?

…………………………………………………………

D. Knowledge, perception and experiences concerning control

20**.** Can the onion bulb rot disease be prevented /controlled? Yes………

No……………

21. If **<u>yes</u>** what do you think can be done to control the spread of the disease?

…………………………………………………………………………………

…………………………………………………………………………………

……………………………………………..

22. What have you done to your onion plants to control the disease?

…………………………………………………………………………………

23. If you have done nothing give reasons

……………………………………………………………………………………

24. Do you practice crop rotation? If yes how many years' intervals do you rotate?

....

E. Knowledge, perception, and experiences concerning economic importance of bulb rotting disease of onion.

25. Expected yield, volume harvested and reasons for yield difference

Year	Farm size	Expected yield	Volume harvested	Reasons for difference
2007				
2008				
2009				
2010				

26. People's reasons for planting onion and what they do when their onion get rotten

Year	Reasons for growing onion	% affected by the disease	What did the people do when plants were affected?
2007			
2008			
2009			
2010			

26. Farmer's contact number…………………………………………………………

APPENDIX 2: Analyses of Variance for onion basal rot disease incidence among three communities (Abuakwa, Mpream Quarters and Manguasi) in the Fanteakwa and Kwahu south districts

Variate: %_Disease incidence

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Farms	4	175.69	43.92	1.26	0.359
Location	2	426.63	213.31	6.14	0.024
Residual	8	278.07	34.76		
Total	14	880.38			

Tables of means

Variate: %_Disaese incidence

Grand mean 16.84

Farms	1	2	3	4	5
	19.51	17.52	21.34	12.97	12.87

LOCATION	Abuakwa	M Quarters	Manguasi
	17.10	10.18	23.24

Least significant differences of means (5% level)

Table	Farms	LOCATION
rep.	3	5
d.f.	8	8
l.s.d.	11.101	8.598

APPENDIX 3: ANOVA for disease incidence and severity Fanteakwa and Kwahu South

(a)

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Fanteakwa	5	17.10	67.36	8.207	3.670
Kwahu_south	5	25.30	34.14	5.843	2.613

Difference of means: -8.202
Standard error of difference: 4.506

95% confidence interval for difference in means: (-18.59, 2.188)

Test of null hypothesis that mean of Fanteakwa is equal to mean of Kwahu_south

Test statistic t = -1.82 on 8 d.f.

Probability = 0.106

APPENDIX 4: ANOVA for disease severity Fanteakwa and Kwahu south

(b)

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Fanteakwa	5	0.560	0.1080	0.3286	0.1470
Kwahu_south	5	1.480	0.4070	0.6380	0.2853

Difference of means: -0.920
Standard error of difference: 0.321
95% confidence interval for difference in means: (-1.660, -0.1799)
Test of null hypothesis that mean of Fanteakwa is equal to mean of Kwahu south

Test statistic t = -2.87 on 8 d.f.

Probability = 0.021

APPENDIX 5: Analysis of variance of effects of *Trichodema asperellum* and *Bacillus subtilis* on radial mycilial growth and sporulation of *Fusarium oxysporium* f.sp.*cepae* in dual culture and incubated in the laboratory at 23 to 31^{o}C and 60 to 70% RH for seven days.

Analysis of variance

Variate: DAY_1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BIOAGENT	1	151.00167	151.00167	9060.10	<.001
Residual	4	0.06667	0.01667		
Total	5	151.06833			

Variate: DAY_2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BIOAGENT	1	2983.74000	2983.74000	2.984E+05	<.001
Residual	4	0.04000	0.01000		
Total	5	2983.78000			

Variate: DAY_3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BIOAGENT	1	6.326E+03	6.326E+03	9.884E+05	<.001
Residual	4	2.560E-02	6.400E-03		
Total	5	6.326E+03			

Variate: DAY_4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BIOAGENT	1	4720.81500	4720.81500	4.721E+05	<.001
Residual	4	0.04000	0.01000		
Total	5	4720.85500			

Variate: DAY_5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BIOAGENT	1	4743.28167	4743.28167	2.846E+05	<.001
Residual	4	0.06667	0.01667		
Total	5	4743.34833			

Variate: DAY_6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BIOAGENT	1	4760.16667	4760.16667	2.856E+05	<.001
Residual	4	0.06667	0.01667		
Total	5	4760.23333			

Variate: DAY_7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BIOAGENT	1	4760.16667	4760.16667	2.856E+05	<.001
Residual	4	0.06667	0.01667		
Total	5	4760.23333			

APPENDIIX 6: ANOVA for seed germination test in the laboratory

Analysis of variance for normal seedlings

Variate: NORMAL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
units stratum					
DAY	1	731.531	731.531	300.12	<.001
REP	3	11.094	3.698	1.52	0.236
CULTIVAR	3	9.094	3.031	1.24	0.316
Residual	24	58.500	2.438		
Total	31	810.219			

Tables of means

Variate: NORMAL

CULTIVAR	B. RED	MALAVI	R. CREOLE	TEXAS
	40.75	39.38	39.62	39.62

Analysis of variance for abnormal seedlings

Variate: ABNORMAL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
units stratum					
DAY	1	612.500	612.500	211.13	<.001
REP	3	5.125	1.708	0.59	0.628
CULTIVAR	3	2.625	0.875	0.30	0.824
Residual	24	69.625	2.901		
Total	31	689.875			

Tables of means

Variate: ABNORMAL

CULTIVAR	B. RED	MALAVI	R. CREOLE	TEXAS
	6.50	6.75	6.88	6.12

Analysis of variance for Dead seeds

Variate: DEAD

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
units stratum					
DAY	1	0.2812	0.2812	0.39	0.540
REP	3	3.3438	1.1146	1.53	0.233
CULTIVAR	3	4.0938	1.3646	1.87	0.161
Residual	24	17.5000	0.7292		
Total	31	25.2188			

Tables of means

Variate: DEAD

CULTIVAR	B. RED	MALAVI	R. CREOLE	TEXAS
	2.75	3.38	3.62	3.62

NOTE Normal seedlings were those expected to survive to complete the live cycle of the plant, abnormal seedlings referred to week once and dead seeds those that fail to germinate.

APPENDIX 7: Analysis of the emergence and survival of onion seedlings planted in soil inoculated with *Fusarium oxysporium* f. sp. *Cepae*

Analysis of variance for seedling emergence

Variate: %_EMERGENCE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	2.04	0.68	0.06	
REP.*Units* stratum					
TREATMENT	1	10616.07	10616.07	887.81	<.001
VARIETY	3	1804.56	601.52	50.30	<.001
TREATMENT.VARIETY	3	1379.92	459.97	38.47	<.001
Residual	21	251.11	11.96		
Total	31	14053.70			

Tables of means

Variate: %_EMERGENCE

Grand mean 69.64

TREATMENT	INOCULATED	NOT INOCULATED
	51.43	87.86

VARIETY	B RED	MALAVI	R CREOLE	TEXAS
	81.07	66.78	70.36	60.36

TREATMENTVARIETY	B RED	MALAVI	R CREOLE	TEXAS
INOCULATED	72.15	45.71	54.29	33.57
NOT INOCULATED	90.00	87.85	86.43	87.14

Analysis of variance for seedling survival

Variate: %_SURVIVAL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	3	144.90	48.30	1.86	

REP.*Units* stratum

TREATMENT	1	15467.33	15467.33	594.48	<.001
VARIETY	3	2027.43	675.81	25.97	<.001
TREATMENT.VARIETY	3	1520.90	506.97	19.49	<.001
Residual	21	546.38	26.02		
Total	31	19706.93			

Tables of means

Variate: %_SURVIVAL

Grand mean 64.26

TREATMENT	INOCULATED	NOT INOCULATED
	42.28	86.25

VARIETY	B RED	MALAVI	R CREOLE	TEXAS
	76.79	60.98	64.28	55.00

TREATMENTVARIETY	B RED	MALAVI	R CREOLE	TEXAS
INOCULATED	65.72	34.82	42.86	25.71
NOT INOCULATED	87.86	87.14	85.71	84.28

APPENDIX 8: Analysis of onion bulb rot disease progress on three onion cultivars (Bawku red Malavi and Red Creole) planted in soil inoculated with *Fusarium oxysporium* f. sp. *Cepae*

Analysis of variance

Variate: WEEK_1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.	0.		
REP.*Units* stratum					
INOCULATED	1	0.	0.		
VARIETY	2	0.	0.		
INOCULATED.VARIETY	2	0.	0.		
Residual	64	0.	0.		
Total	71	0.			

Variate: WEEK_2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.	0.		
REP.*Units* stratum					
INOCULATED	1	0.	0.		
VARIETY	2	0.	0.		
INOCULATED.VARIETY	2	0.	0.		
Residual	64	0.	0.		
Total	71	0.			

Variate: WEEK_3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.0278	0.0139	0.10	
REP.*Units* stratum					
INOCULATED	1	5.5556	5.5556	40.38	<.001
VARIETY	2	0.0278	0.0139	0.10	0.904
INOCULATED.VARIETY	2	0.0278	0.0139	0.10	0.904
Residual	64	8.8056	0.1376		
Total	71	14.4444			

Variate: WEEK_4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.0833	0.0417	0.31	
REP.*Units* stratum					
INOCULATED	1	6.1250	6.1250	45.23	<.001
VARIETY	2	0.0000	0.0000	0.00	1.000
INOCULATED.VARIETY	2	0.0000	0.0000	0.00	1.000
Residual	64	8.6667	0.1354		
Total	71	14.8750			

Variate: WEEK_5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.3611	0.1806	0.93	
REP.*Units* stratum					
INOCULATED	1	8.6806	8.6806	44.84	<.001
VARIETY	2	0.4444	0.2222	1.15	0.324
INOCULATED.VARIETY	2	0.4444	0.2222	1.15	0.324

Residual	64	12.3889	0.1936		
Total	71	22.3194			

Variate: WEEK_6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.1944	0.0972	0.78	
REP.*Units* stratum					
INOCULATED	1	16.0556	16.0556	128.89	<.001
VARIETY	2	5.8611	2.9306	23.53	<.001
INOCULATED.VARIETY	2	5.8611	2.9306	23.53	<.001
Residual	64	7.9722	0.1246		
Total	71	35.9444			

Variate: WEEK_7

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.0278	0.0139	0.08	
REP.*Units* stratum					
INOCULATED	1	23.3472	23.3472	133.15	<.001
VARIETY	2	9.5278	4.7639	27.17	<.001
INOCULATED.VARIETY	2	9.5278	4.7639	27.17	<.001
Residual	64	11.2222	0.1753		
Total	71	53.6528			

Variate: WEEK_8

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.3611	0.1806	0.60	
REP.*Units* stratum					
INOCULATED	1	34.7222	34.7222	115.11	<.001
VARIETY	2	15.4444	7.7222	25.60	<.001
INOCULATED.VARIETY	2	15.4444	7.7222	25.60	<.001
Residual	64	19.3056	0.3016		
Total	71	85.2778			

Variate: WEEK_9

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.3611	0.1806	0.60	
REP.*Units* stratum					
INOCULATED	1	34.7222	34.7222	115.11	<.001
VARIETY	2	15.4444	7.7222	25.60	<.001
INOCULATED.VARIETY	2	15.4444	7.7222	25.60	<.001
Residual	64	19.3056	0.3016		
Total	71	85.2778			

Variate: WEEK_10

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.6944	0.3472	1.27	
REP.*Units* stratum					
INOCULATED	1	42.0139	42.0139	153.16	<.001
VARIETY	2	15.3611	7.6806	28.00	<.001
INOCULATED.VARIETY	2	15.3611	7.6806	28.00	<.001
Residual	64	17.5556	0.2743		
Total	71	90.9861			

APPENDIX 9: Analysis of onion bulb rot disease severity on three onion cultivars (Bawku red Malavi and Red Creole) planted in soil inoculated with *Fusarium oxysporium* f. sp. *Cepae*

Analysis of variance

Variate: SUM_AUDPC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	84.0	42.0	0.21	
REP.*Units* stratum					
TREATMENT	1	53165.2	53165.2	271.41	<.001
VARIETY	2	11923.7	5961.8	30.44	<.001
TREATMENT.VARIETY	2	11923.7	5961.8	30.44	<.001
Residual	64	12536.5	195.9		

Total	71	89633.1

Tables of means

Variate: SUM_AUDPC

Grand mean 90.2

TREATMENT	CONTROL	INOCULATED
	63.0	117.3

VARIETY	B. Red	Malavi	R. C
	108.2	79.0	83.3

TREATMENT	VARIETY	B. Red	Malavi	R. C
CONTROL		63.0	63.0	63.0
INOCULATED		153.4	95.1	103.5

APPENDIX 10: Analysis of effect of FOC on weight of onion bulbs formed

Variate: YIELD_wt/g

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	110.3	55.1	0.35	
REP.*Units* stratum					
VARIETY	2	2217.4	1108.7	7.14	0.002
Residual	67	10410.5	155.4		
Total	71	12738.1			

Tables of means

Variate: YIELD

Grand mean 34.9

VARIETY	B. Red	Malavi	R. Creole
	28.0	41.6	35.1

APPENDIX 11: Components of microprep buffers

DNA extraction buffer	2.5 parts
Nuclei lysis buffer	2.5 parts
5% sarkosyl	1.0 part
0.5 g sodium bisulfate	100 mls